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Human Enteric Virus Survival in Soil Following Irrigation with Sewage Plant Effluents

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16. ABSTRACT

The wastewater treatment processes at Kerrville and Uvalde, Texas, were evaluated in terms of their efficacy in reducing human enteric viruses. (Data on the reduction of TOC, BOD_5, suspended solids, orthophosphate, nitrogenous compounds, total coliform, fecal coliform, and bacteriophage were also obtained.) Enteric viruses were reduced by greater than 99% at Kerrville and at least 99% at Uvalde. These waters are used for irrigation without disinfection. Soil samples at the Kerrville and Uvalde application sites yielded both fecal coliforms and bacteriophages. In addition, two confirmed enterovirus isolations were made at the Kerrville site. Lysimeters placed 1.5 ft, 3.0 ft, and 4.5 ft depths at the Kerrville site yielded large numbers of bacteriophage isolates. In addition, ten lysimeter samples yielded a total of 29 confirmed viral isolates. This is a strikingly high number of isolations of indigenous enteric viruses, relative to the irrigation pond which was demonstrably low in viruses (when assayed on the same cell lines). Cell changes (CPE) but no confirmed isolations were made from five monitoring wells.

These studies of wastewater treatment plants processing dilute to moderate strength sewage in efficient treatment schemes represent a "best possible case" for the use of undisinfected, domestic wastewater effluents for irrigation. The isolation of enteroviruses in water from lysimeters but not from the monitoring wells suggests that depth to groundwater should be a critical factor in the selection of irrigation sites. From data developed in this study, it appears that a depth of 4.5 ft is not sufficient for effective viral attenuation in soils such as those described in this report.

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HUMAN ENTERIC VIRUS SURVIVAL IN SOIL FOLLOWING IRRIGATION WITH SEWAGE PLANT EFFLUENTS

by

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FOREWORD

The U. S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low level insults.

This report provides an evaluation of the fate of naturally-occurring viruses in undisinfected treated sewage effluents applied to the soil through irrigation. Sewage treatment removes many but not all viruses from the effluent. Therefore, a knowledge of the survival and fate of these viruses is important in assessing the health risk to farm workers and consumer of food crops and ground water in areas where domestic wastes are applied to the land. This study has provided a significant contribution to the data base required to fully assess the health considerations of this waste disposal practice.

R. J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

The wastewater treatment processes at Kerrville and Uvalde, Texas, have been evaluated in terms of their efficacy in reducing TOC, BOD_5, suspended solids, orthophosphate, nitrogenous compounds, total coliform, fecal coliform, bacteriophage and human enteric viruses. The Kerrville facility, an underloaded parallel oxidation ditch-trickling filter system, is highly effective; achieving a 90-95% reduction in BOD_5 and an 80% reduction in TOC. By comparison, the Uvalde plant, utilizing an overloaded trickling filter followed by a series of six ponds, reduces incoming levels of BOD_5 and TOC by 85% and 75%, respectively. Both plants reduce phage levels by a factor of 300 to 500. Enteric viruses are reduced by greater than 99% at Kerrville and at least 99% at Uvalde. These waters are used for irrigation without disinfection.

Soil samples at the Kerrville and Uvalde application sites have yielded both fecal coliforms and bacteriophages. In addition, two confirmed enterovirus isolations were made at the Kerrville site.

The Kerrville lysimeters (at 1.5 ft, 3.0 ft, and 4.5 ft depths) have yielded large numbers of bacteriophage isolates. In addition, ten lysimeter samples yielded a total of 29 confirmed viral isolates. This is a strikingly high number of isolations of indigenous enteric viruses, relative to the irrigation pond which was demonstrably low in viruses (when assayed on the same cell lines).

Monitoring wells at the Kerrville site show water quality to be improved over that in the irrigation pond. Nitrate values are somewhat elevated, however. Further, indicator organisms (fecal coliform and fecal streptococci) were isolated from all wells at some time and with relatively high frequency (not less than 40% of sampling times from any well). No confirmed virus isolations were made from any of the monitoring wells.

Geological descriptions of the site are included as are well log and hydrologic analyses.

These studies of wastewater treatment plants processing dilute to moderate strength sewage in efficient treatment schemes represent a "best possible case" for the use of undisinfected, domestic wastewater effluents for irrigation. The isolation of enteroviruses in water from lysimeters but not from the monitoring wells suggests that depth to groundwater should be a critical factor in the selection of irrigation sites. From data developed in this study, it appears that a depth of 4.5 ft is not sufficient for effective viral attenuation in soils such as those described in this report.

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ABBREVIATIONS

BGM -- Buffalo Green Monkey

BOD_5 -- biochemical oxygen demand-5 day

C -- centigrade

CEC -- cation exchange capacity

cfu -- colony forming units

cm -- centimeter

COD -- chemical oxygen demand

COLE -- coefficient of linear expansion

CPE -- cytopathic effect

fm -- formation

g -- gravity

gm -- grams

gpd -- gallons per day

gpm -- gallons per minute

l -- liter

meq -- milliequivalents

MF -- membrane filtration

mg -- milligram

mgd -- million gallons per day

ml -- milliliter

MLSS -- mixed liquor suspended solids

mm -- millimeters

MPN -- most probable number

msl -- mean sea level

ND -- none detected

PCB -- polychlorinated biphenols

PEG -- polyethylene glycol

pfu -- plaque forming units

pH -- hydrogen ion concentration

ppm -- parts per million

psi -- pounds per square inch

PVC -- polyvinyl chloride

rpm -- revolution per minute

TKN -- total Kjeldahl nitrogen

TOC -- total organic carbon

TPO_4 -- tryptose phosphate broth

TSS -- total suspended solids

VSS -- volatile suspended solids

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Above all, we are grateful to Dr. Elmer Akin for his close review of this project, for his many astute observations and for his perspicacious--even trenchant--criticisms. By these means, he has contributed greatly.

SECTION 1

INTRODUCTION

Sewage farming has been used widely in the Far East for centuries and in Europe for over one hundred years. While practiced in the past on a limited scale in the United States, land application of wastewaters now has become attractive from both an environmental and economic perspective. Further, its popularity has been enhanced by recent amendments to the Federal Water Pollution Control Act (PL 92-500).

Increased interest in the public health concerns associated with the land application of wastewaters has paralleled the popularity of the process. Of particular public health concern is the possible survival and accumulation of pathogens in the soil and their movement to groundwater supplies. An urban treatment plant receives significant quantities of Clostridia, *Enterobacter, Klebsiella, Leptospira,* Mycobacteria, *Proteus, Providencia, Staphylococci,* as well as human enteroviruses. The degree to which these are passed on through the plant to land application sites is a function of the efficiency of the wastewater treatment chain prior to application to soils.

Pathogen movement through soils has been reported for distances up to 1500 feet. Release and subsequent movement of bacteria is not unexpected as this is a reversible phenomenon and, at least in part, ion dependent. The same theoretical considerations appear to govern viral movement through soils. Fewer data are available in the literature for viruses than for bacteria because of the problems inherent in their apparent lesser frequency of occurrence and the nature of concentration and assay techniques (which, in turn, may account for the reported lower frequency).

Because more municipalities are turning to land application, especially where economically attractive, the potential for groundwater pollution is increasing. The degree of that potential is not known. The studies carried out under this grant were designed to evaluate the survival and transport of human enteric viruses through the soil at an operating land application site in South Central Texas. Although it focuses specifically on human enteroviruses, it seeks to corroborate and place in perspective the results by concomitant analyses for bacterial indicators, bacteriophages and selected chemical parameters. Samples of raw wastewater, treated effluent, holding pond water, soils, water from the zone of percolation and from the underlying aquifer have been analyzed. Only under field conditions can the potential pollution hazard be studied meaningfully.

SECTION 2

CONCLUSIONS

As essentially all of the field effort undertaken during this study was directed toward the Kerrville site, the conclusions drawn are based primarily on observations made at that site. Specific information regarding the Uvalde site can be found in the text of this report.

Geological descriptions of the wastewater application site indicate that it is typical of the South Central Texas area with its limestone formations. Hydrologic studies indicate that groundwater moves toward Third Creek in a southerly direction across the irrigation site. It is either discharged to the creek as seeps or continues as subsurface flow in the alluvium down-valley.

The treatment processes are effective in treating moderate strength domestic wastewater. The resulting effluent is moderately low in coliforms and bacteriophages and low in enteric viruses. Despite this, there were isolations of fecal coliforms, bacteriophage and enteric viruses from soil and lysimeter samples in significant numbers. The levels of phage found in lysimeters, even to a depth of 4.5 ft, were remarkably high. No strong evidence for attenuation by passage through soils up to 4.5 ft could be found either during the routine monitoring or in the special lysimeter studies which utilized an intensive irrigation regimen. In addition, positive isolations of enteric viruses from lysimeter samples were made and the viruses were identified. In view of the low levels of enteroviruses detected in the irrigation pond, a conservative role of soil in the survival of phages and enteric viruses is suggested.

The monitoring wells yielded total coliforms, fecal coliforms, and fecal streptococci with relatively high frequency. Median fecal coliform levels were from 2.4×10^{0} to 3.7×10^{2} cfu/100 ml while median fecal streptococci values were from 6.7×10^{-1} to 7.7×10^{1} cfu/100 ml. No monitoring well had fewer than 40% of samples positive for fecal coliform, or 39% positive for fecal streptococci. No confirmed virus isolations were made from any of the monitoring wells.

It was not possible to calculate a mass balance of indigenous enteroviruses at the site due to low numbers of enteroviruses observed at most of the sampling points. In general, the Kerrville site represents a well-operated, biologically and chemically efficient treatment system (including the land application component). In some respects, the efficiency of this system precluded achieving this particular stated objective of the study.

These studies of wastewater treatment plants processing dilute to moderate strength sewage in efficient treatment schemes represent a "best possible case" for the use of undisinfected, domestic wastewater effluents for irrigation. The isolation of enteroviruses in water from lysimeters but not from the monitoring wells suggests that depth to groundwater should be a critical factor in the selection of irrigation sites. From data developed in this study, it appears that a depth of 4.5 ft is not sufficient for effective viral attenuation in soils such as those described in this report.

In general, the land application of wastewaters with as low an enterovirus density as that observed at the study site onto soils such as those described for the Ke[illegible]ille site appears to provide adequate attenuation of enteroviruses, and should not represent an undue hazard to any potential, beneficial uses of groundwater or surface water.

SECTION 3

RECOMMENDATIONS

In general, the land application of wastewaters with as low an enterovirus density as that observed at the study site onto soils such as those described for the Kerrville site appears to provide adequate attenuation of enteroviruses, and should not represent an undue hazard to any potential, beneficial uses of groundwater or surface water.

The isolation of enteroviruses from lysimeters remains a disturbing finding, however. In particular, the recovery of polioviruses lacking temperature sensitivity (ts) is of some concern. It would be useful, in assessing the meaning of such data, if the proportion of poliovirus isolates lacking this ts marker were known for virions found in raw influent and in treated effluents. One would wish to determine whether vaccine strains and those lacking the ts marker survive the irrigation process and soil percolation equally. Further, more critical genetic identification of isolated viral strains should be made. It is essential that field isolates be shown incontrovertibly to be different from reference strains used in laboratories carrying out such studies. Thus, it is recommended that such techniques as RNA fingerprinting be developed for representative enteroviruses and applied to the differentiation of field isolates from laboratory strains.

In addition, because soil samples yielded fewer positive viral isolations than did lysimeter water samples, the suspicion remains that the existing methodology for viral recovery from soils is simply inadequate. More effort should be devoted to improving recovery methodology from field samples; especially from soils, pond sediments, and algal-rich waters.

Other investigations of this nature should be conducted at field sites which are significantly different in terms of wastewater quality, soils and groundwater hydrology. It certainly would appear important and prudent to evaluate a "worst case" site in terms of the results reported herein. However, it will be of utmost importance that similar methodologies be used in order to make a comparative analysis of the data.

SECTION 4

BACKGROUND

INTRODUCTION

The first organized efforts at sewage farming in England and in continental Europe date from the mid-nineteenth century. Sanitary conditions deteriorated rapidly as the Industrial Revolution brought large numbers of people from sparsely settled rural regions into densely populated and often-times ill-planned urban areas. The most critical problems of waste purification first developed in England. The dilution and purification capacities of her smaller rivers were exceeded as increasing amounts of sewage reached these waterways.

The first Royal Commission on Sewage Disposal in England was appointed to study and report on sewage problems in 1857 (Chase, 1964). Their report, filed in 1865, recommended that distribution of sewage on land was a proper method of purification and the only means of preventing pollution of rivers. Subsequent commissions appointed in 1868 and 1882 further defined the options of sewage collection and disposal and returned similar recommendations.

The practical application of land treatment proved to be difficult due to the nonporous nature of certain soils and the relatively large amounts of land necessary for the spreading of sewage. Sewage farms in Paris in 1924 covered 12,584 acres (Chase, 1964), while a similar operation for the City of Berlin utilized 56,800 acres in 1934 (Gray, 1968).

The direct application of sewage to land for the fertilization of crops was never carried out with continuous success in the United States. However, sewage irrigation with effluent from wastewater treatment plants has been practiced in arid and semiarid regions of the country. In 1968, approximately 60 municipalities on the High Plains of West Texas were diverting treated effluent to the land (Wells, 1968). Ideally, the application of wastewaters in this manner serves the multiple purposes of crop irrigation, effluent disposal, and groundwater recharge in areas where surface water is scarce.

Surface spreading in basins or ditches, irrigation of land, and stream impoundment is most successful (in terms of amount of water recharged) where water table aquifers exist. However, for the same reasons, these unconfined aquifers are more susceptible to surface pollution, especially in those regions where the water table is near ground level. Mechanically, surface spreading is accepted as most feasible where lower quality water is available, as suspended solids can be removed by filtration through the bottom of the spreading area. Other methods of application of wastewater include spray or trickle irrigation and overland flow. Operation and design of these type systems have been described previously (Pound and Crites, 1973; and Reed et al, 1972).

Regardless of the methods chosen to treat and dispose of effluents, the persistent question of water quality arises. It is an inevitable fact that possible contamination of groundwater is inherent in any recharge system. The probability of introducing pollutants is increased if wastewater in some form is used for recharge purposes. The consequences of groundwater contamination can be damaging because of the possibility of long-term persistence even after the contaminating source has been eliminated.

An optimized sewage irrigation system would allow the continuous application of wastewater to a relatively small surface area, with only minimal pretreatment necessary to alleviate aesthetic problems. After percolating through the unsaturated soil zone, the resulting groundwater would be suitable for reuse without further treatment (McGauhey and Krone, 1967). Unfortunately, nature has placed physical, chemical, and biological constraints upon such an idealized system. Detailed discussions in this regard have been provided by Sepp (1971) and Sorber and Guter (1975).

Physical Limitations

Physical clogging of the soil pore space results in the loss of infiltration rates. Such "sewage-sick" soils have led to the failure of many wastewater irrigation systems. Some of the factors related to the cause of clogging have been identified as compaction of soil, deflocculation of soils (resulting in decreased permeability when sodium represents a high percentage of the cationic content of the water), deposition of suspended solids, and growth of bacterial slimes especially under anaerobic conditions which allow the precipitation of ferrous sulfide (McGauhey and Krone, 1967). Experience has shown that the most severe clogging problems can be eliminated by restoring aerobic conditions within the soil system by controlling the frequency of effluent application (Thomas and Law, 1968).

Chemical Limitations

The behavior of various chemicals prevalent in domestic wastewaters has been studied at various irrigation sites (Pound and Crites, 1973; Sorber and Guter, 1975). Traditionally, the presence of heavy metals and synthetic organic chemicals is attributable to industrial discharges and urban runoff. This report will not emphasize such studies, concentrating instead on biological hazards. The literature documenting removal of either of these chemical classes by secondary treatment is limited. However, trace metals are associated with sludge solids. Nomura and Young (1974) reported that 90% of aluminum, iron, mercury, lead, and zinc settled with the biomass when suspended solids removals were 90-95%. Under the same conditions, chromium (VI) and nickel had median removals of 77% and 50%, respectively. Conversely, no significant removal of a wide variety of potentially carcinogenic organic compounds at three activated sludge treatment plants was observed (Malaney et al, 1967). Using routine analytical procedures, however, all chlorinated organics examined for in dry sludges were found (see Table 1).

Clearly, the concentration of trace metals in sludges and effluents is a primary consideration in the land disposal of these residuals. While arsenic, copper, lead, mercury, nickel, selenium, and zinc are of concern, cadmium poses the greatest human health problem because of its accumulation in kidneys and liver and its subsequent toxic effects beyond a threshold level. As trace elements are retained in soils, their entry into the food chain and their long-term accumulation in soil systems present potential health risks. Hinesly et al, (1977) have discussed the effects of sewage sludge applications on assimilation of zinc and cadmium by a typical feed crop, corn. Differential levels of cadmium and zinc have been shown in fruit and leaves of corn, soy beans and tomatoes (Kirkham, 1974; Table 2). At this time, however, no apparent consensus exists among technical experts and regulatory agencies as to what the toxicity levels are. These problems are compounded further by inadequate methodology for the evaluation of toxicological and epidemiological effects of heavy metals in the environment.

Much the same argument can be made for the ultimate fate of a seemingly endless number of synthetic organic chemicals found in wastewater effluents and sludges. Studies have shown translocation of chlordane, heptachlor, and dieldrin from soil into the oil of soybean seeds (Moore et al, 1976b). While the amounts detected were low, these findings demonstrate that sludge can serve to recycle organic contaminants back into the food chain.

TABLE 1. Pesticide and PCB Content, Dry Sludge[α]

Contaminant	Range (ppm) Min.	Range (ppm) Max.	Sludges Examined
Aldrin*	ND	16.2	5
Dieldrin*	0.08	1.4	7
Chlordane*	3.0	32.2	7
DDT+DDD*	0.1	0.1	7
PCB's[+]	ND	352.0	69

[α] From Jelinek et al, 1976.

[*] Examined, 1971.

[+] Examined, 1971, 1973, 1975.

TABLE 2. Concentration of Trace Elements in Plants*

Plant	Cd (ppm) Fruit	Cd (ppm) Leaves	Zn (ppm) Fruit	Zn (ppm) Leaves
Corn	1.03	11.6	152.3	212
Soybeans	2.40	10.2	80.0	249
Tomatoes	0.50	6.1	29.0	153

* From Kirkham, 1974.

Studies in Lubbock, Texas, showed a marked deterioration of chemical quality in the aquifer after sustained irrigation with effluent (Wells, 1968). While BOD and COD were eliminated, calcium, magnesium, chloride, sulfate, and nitrates increased significantly. Nitrate levels in the water beneath the storage pond on the Texas Tech University farm were increasing at a rate of approximately 1.0 mg/l/month.

More detailed information regarding the interaction of vegetation in a soil system was reported from the Penn State Wastewater Renovation and Conservation Project where municipal effluent was applied to both forest and cropland. Phosphorus was retained effectively within the first six to 24-inch soil horizon. Calculations based on experimental data showed that only 1.7 percent of the phosphorus added yearly passed the four-foot depth (Hook, *et al*, 1973). The remaining 98.3% was removed either by the harvest of reed canary-grass, by fixation in the soil, or by surface runoff. The cycling of nitrogen applied in effluent within the soil system led to increased nitrate levels. The concentration of nitrate-nitrogen was held below 10 mg/l (USEPA limit for drinking water) at the four-foot soil depth by grass-legume hays and reed canary-grass. Forested areas rapidly exceeded USEPA drinking water standards reaching 25 to 40 mg/l nitrate-nitrogen at four feet below the surface with the application of two inches of effluent per week (Kardos and Sopper, 1973a). It is apparent, therefore, that the potential for microbial denitrification must be maximized in any continuous irrigation project.

Changes in other chemical constituents at the Penn State irrigation site demonstrated the removal of potassium, calcium, magnesium, sodium, manganese, boron, and chlorides from the wastewater effluent at the four-foot depth. In the crop rotation plots, increases in the soil content of sodium, magnesium, boron and chlorides were observed (Kardos and Sopper, 1973b). Such accumulations of soluble salts are much more likely to occur in those regions where effluent provides the major source of irrigation water and rainfall is relatively low. Accumulations of salts and heavy metals could present the dual problems of reducing soil permeability and of increasing toxicity towards plants.

Biological Limitations

Perhaps one of the greatest constraints placed upon sewage irrigation is the survival and movement of pathogens. Even before the final acceptance of the germ theory of disease, John Snow published results linking London's outbreaks of Asiatic cholera with an underground contaminated water supply (Burrows, 1968). In like fashion, the classic work of Neefe and Stokes (1945) on infectious hepatitis was founded initially on epidemiological evidence which implicated sewage-contaminated well water as the source of infection.

The concentration of biological contaminants in domestic sewage is influenced by several complex factors including the age and health of the contributing population as well as the season of year. Species of potentially pathogenic microorganisms may be categorized conveniently as bacteria, helminthic parasites, protozoa, and viruses. The presence and survival of these major groups of pathogens in wastewater were reviewed by Foster and Engelbrecht (1973) and Akin, et al (1978). While certain pathogenic organisms such as *Salmonella typhi* have a relatively brief survival time in wastewater, other pathogens (including mycobacteria, *Ascaris* ova, and certain enteric viruses) are highly resistant to many environmental stresses.

The levels of microbial pathogens ultimately present in sewage effluents or sludges depends upon the degree of removal achieved by the treatment train employed. Results given in Table 3 indicate that primary treatment alone does not remove the pathogen load in domestic wastewater, although primary sludges may contain large numbers of parasite eggs. On the other hand, the sludge biomass generated by conventional secondary treatment may be expected to contain a large portion of that microbial population which has been removed from incoming wastewater.

TABLE 3. Pathogen Removal by Conventional Wastewater Treatment (%)*

Organism	Primary Sedimentation	Trickling Filters	Activated Sludge
Salmonella	15	84-99+	96-99
Mycobacterium	48-57	69-99	Slight-87
Amoebic Cysts	No Reduction (3 hr)	11-99+	No Apparent Removal
Helminth Ova	72-98	62-76	No Apparent Removal
Enteric Viruses	3-extensive	0-84	76-99

* From Foster and Engelbrecht, 1973.

Generally, disinfection (specifically chlorination) of effluents before discharge provides the last step in the treatment scheme. Difficulties arise in comparing results of chlorination studies because of a lack of specific information on (1) initial chlorine dosage, (2) amount and form of chlorine residual, (3) temperature, (4) pH, and (5) the presence of organic or inorganic nitrogenous compounds. However, certain microorganisms including mycobacteria, amoebic cysts, some enteric viruses, and the viral agent of waterborne hepatitis, may be more chlorine-resistant than are indicator coliform organisms. Nevertheless, chlorination of effluents can provide an additional removal of certain potential pathogens as illustrated by field studies in Table 4.

TABLE 4. Effect of Wastewater Treatment on Various Organisms*

	Total Reduction in Treatment Plant (Log_{10})	Reduction by Chlorination Only (Log_{10})
Plant #1 (Standard-rate trickling filter, chlorine residual 1.5 mg/l)		
Fecal coliform	3.7	2.1
Fecal streptococci	2.4	2.0
Salmonella[+]	1.4	0.5
Enteric viruses[α]	0.4	0.3
Plant #2 (High-rate trickling filter, chlorine residual 4 mg/l w/improved mixing)		
Total coliform	6.8	5.5
Fecal coliform	5.8	3.6
Klebsiella	5.2	3.7

* From Sorber et al, 1974.

+ Presumptive, M-bismuth sulfite broth with MF procedure

α Three days on BGM cells after concentration

A typical microbial analysis of a sample from the aeration chamber of an urban treatment plant (Table 5) yielded confirmed isolates of species of Clostridia, *Enterobacter*, H_2S-producing *Escherichia*, fecal coliform, *Klebsiella*, *Leptospira*, mycobacteria, *Providencia*, staphylococci; total coliforms in the sample were $1.1x10^8$/100 ml and total plate count was $5.8x10^8$/100 ml (Guentzel, 1978). Concentration of the microbial population into the biomass makes it essential, therefore, to implement sludge processing procedures which can diminish the concentrations of bacteria, helminth ova, and viruses.

TABLE 5. Results of Wastewater Microbiological Screen

Bacteria:	cfu/100 ml	
Citrobacter	$<5.0x10^4$	(ND)
Clostridium	$2.8x10^2$	
Edwardsiella	$<5.0x10^4$	(ND)
Enterobacter	$3.0x10^6$	
Escherichia (H_2S^+)	$1.0x10^6$	
Fecal Coliform	$1.0x10^7$	
Klebsiella	$6.0x10^6$	
Leptospira	$4.6x10^3$	
Mycobacteria	$7.0x10^4$	
Providencia	$1.0x10^6$	
Serratia	$<5.0x10^4$	(ND)
Staphylococcus	$3.0x10^5$	
Total coliform	$1.1x10^8$	
Total Plate Count	$5.8x10^8$	
Yersinia	*	(ND)

Of the total number of colonies which were randomly picked and biochemically tested for Enterobacteriaceae:

Oxidase positive	44.9%
Enterobacter	14.4%
Klebsiella	14.4%
Proteus	1.4%
Providencia	1.4%
Escherichia	1.4%
No growth	4.3%
Not identified	17.3%

Note: ND - None Detected
* - Nonquantitative Procedure
+ - From Guentzel, 1978

Anaerobic sludge digestion, aerobic sludge stabilization and sludge lagooning are among the most economically popular methods for treating waste sludges prior to land disposal. The EPA report (Process Manual, 1974) on microbial survival during anaerobic digestion showed that while coliform populations are reduced greatly, other pathogens such as Mycobacteria and *Ascaris* ova can withstand prolonged digestion. Recently, several laboratory studies have reported the fate of selected viruses during anaerobic digestion. Using a fill-and-draw model digester at 35C, Bertucci and coworkers (1975) observed inactivation rates ranging from 74.9% per day for Echovirus II to 97% per day for Coxsackievirus A-9. Ward and Ashley (1976) added poliovirus to digested sludge at 28C and observed an inactivation rate of one $\log_{10}$ per day. In contrast to these findings, Sanders et al, (1979), have reported much slower rates of 0.3 $\log_{10}$ per day using solids-incorporated poliovirus (an important methodological difference) in a 34C reactor. While significant pathogen reductions can be achieved by anaerobic digestion, actual field digesters may be less efficient due to the continuous input of contaminated raw sludges coupled with probable short-circuiting and incomplete mixing.

Aerobic sludge digestion may be viewed as a continuation of the extended aeration process. To date, little information is available dealing directly with pathogen removal by aerobic digestion. It is obvious that some degree of pathogen inactivation will occur during the 15 to 20 day period required for endogenous respiration. Comprehensive studies on the fate of microbial pathogens in sludge lagoon systems also are lacking. Until such time as more definitive data are available, one should assume conservatively that wasted sludges from these systems also will contain environmentally-resistant microbial forms such as mycobacteria, helminth ova, and viruses.

LAND APPLICATION OF WASTEWATER

If domestic sewage is subjected to adequate secondary treatment followed by disinfection, the resultant effluent may be expected to contain on a per unit basis low levels of potential human pathogens. The continual application of effluents to soils may allow the retention and accumulation of such organisms within a given soil profile. The dimensions of the problems inherent in land disposal of effluents have been suggested by Foster and Engelbrecht (1973) as they attempted to relate treatment effectiveness to application rate (organisms/acre/day) for land disposal systems (Table 6).

By using estimated pathogen removal efficiencies of primary and secondary treatment followed by disinfection, Foster and Engelbrecht (1973) computed the number of organisms applied per acre per day at an irrigation rate of two inches per week. Under the stated conditions, 1.6×10^4 viruses, 3.9×10^3 *Salmonella*, 1.2×10^2 mycobacteria, 9.3×10^1 *E. histolytica*, and 3.9×10^1 helminth ova per acre would be introduced into the soil by effluent irrigation. The ultimate fate of these pathogens then would depend upon their survival and movement in any given terrestrial system.

While their calculations included disinfection as an integral part of the treatment train, it should be noted that operational land disposal sites

TABLE 6. Estimated Wastewater Pathogens Applied to Soil*

Pathogen	Raw Wastewater	Number of Organisms per million gallons Primary Effluent	Secondary Effluent	Disinfection+	Organisms applied per acre per day$^{\alpha}$
Salmonella	2×10^{10}	1×10^{10} (50%)‡	5×10^{8} (95%)‡	5×10^{5}	3.9×10^{3}
Mycobacterium	2×10^{8}	1×10^{8} (50%)	1.5×10^{7} (85%)	1.5×10^{4}	1.2×10^{2}
E. histolytica	1.5×10^{7}	1.3×10^{7} (10%)	1.2×10^{7} (10%)	1.2×10^{4}	9.3×10^{1}
Helminth ova	2.5×10^{8}	2.5×10^{7} (90%)	5×10^{6} (80%)	5×10^{3}	3.9×10^{1}
Virus	4×10^{10}	2×10^{10} (50%)	2×10^{9} (90%)	2×10^{6}	1.6×10^{4}

* From Foster and Engelbrecht, 1973.

+ Conditions sufficient to yield a 99.9% kill

α Applied at a rate of 2 inches per week.

‡ Estimated percentage removal efficiency of the treatment.

may use unchlorinated effluents pumped directly from holding ponds, especially in small communities with older installations. These effluents may carry an even larger pathogen load into a soil system.

In similar fashion, residual sludges can transport large quantities of potentially pathogenic organisms onto a land disposal site. Assuming $1x10^3$ enteric viruses per liter MLSS and a solids level of 0.2 to 0.4%, one can estimate the potential transport of human enteric viruses to land disposal sites. Even with a hypothetical 99% reduction in virus level in the anaerobic digestion process, the use of 10 dry tons/acre/year as soil additive implies the addition of more than $2x10^7$ pfu/acre (or about $1x10^3$ pfu/ft, assuming injection or plowing to a six inch depth). The ultimate public health importance of these and other organisms in the soil environment would depend both upon their survival rate and their potential for movement to surface or groundwaters.

Microbial Survival and Transport in Soil Systems

In a recent review, Gerba et al, (1975), listed the factors affecting the survival of enteric bacteria in soil as moisture content, moisture-holding capacity, temperature, pH, sunlight, organic matter, and antagonism from soil microflora. These parameters should be remembered when comparing microbial survival data among various studies. Early studies by Beard (1938, 1940) demonstrated that *Salmonella typhi* could be recovered from loam and peat soils for periods up to 85 days, while survival of this organism in drying sand was only 4 to 7 days. Additionally, *S. typhi* may survive as long as two years at freezing temperatures. Mycobacteria, because of their high content of waxy substances, can survive even dry conditions for long periods of time. Greenberg and Kupka (1975) in a review of available literature cited survival times ranging from 150 days to 15 months for Mycobacteria in soil.

Survival of viruses in soils are influenced by many of the same parameters described above, although at this time little direct evidence supports viral inactivation by antagonistic microorganisms. The effect of temperature on the survival of poliovirus 1 (Chat) is shown in Table 7. As expected, lower temperatures favor longer survival times. Observation of a one $\log_{10}$ loss of viral titer required approximately 3 months at 4C, 1 month at 20C, and less than one week at 30C. In like fashion, an optimal soil moisture content favors poliovirus survival in soil, while dessication results in a more rapid loss of virus recoverability (Table 8). Bagdasaryan (1964), working with a wide variety of human enteroviruses including polioviruses, Coxsackieviruses, and Echoviruses, reported survival times ranging from 110 days to 170 days at a soil pH of 7.5 and a soil temperature of 3 to 10C.

Removal of bacteria from liquid percolating through a soil is due to both mechanical removal (straining or sieving at the soil surface) and adsorption to soil particulates. Studies in Rumania (recently reviewed by Gerba et al, 1975) using coliform bacteria labeled with radioactive phosphorus demonstrated that 92 to 97% of the bacteria were retained in the first centimeter of soil, while 3 to 5% were detected at depths between 1 and 5 cm. The direct relationship of coliform removal from percolating water to increasing cation concentration and decreasing pH are consistent with classical adsorption theory.

TABLE 7. Effect of Temperature on the Survival of Poliovirus I in Soils at 15% Moisture Content

Days	Virus Recovered (%) 4C	 20C	 30C
1	74	99	33
3	68	139	17
8	48	44	2
10	68	40	1
14	47	53	0.5
21	45	24	0.1
28	33	12	0.01
42	22	9	0.006
49	13	5	ND+
80	12	0.7	ND
100	8	0.4	ND
134	5	0.2	ND

* From Duboise et al, 1976a.

+ No virus detected in eluate.

TABLE 8. Effect of Soil Moisture on the Survival of Poliovirus I at 20C*

Days	% Virus Recovered at Various Moisture Contents 25%	15%	Drying⁺
1	69	99	74
3	41	138	35 (10.9)
8	22	44	0.3 (6.2)
10	17	40	0.08 (5.5)
14	13	53	0.02 (4.6)
21	10	24	ND[α]
28	5	12	0.003 (4.6)
42	2	9	ND
49	1	5	0.002 (4.6)
80	0.2	0.7	ND
100	0.07	0.4	ND
134	0.004	0.2	ND

* From Duboise _et_ _al_, 1976a.

+ Sample permitted to dry. Actual moisture content shown in parenthesis.

α No virus detected in eluate.

Bacterial movement through soils has been demonstrated at several field sites. Reporting from the available literature, Gerba et al, (1975) noted coliform movements in a variety of soils for distances ranging from 3 to 1500 feet. Release and movement of microorganisms would be expected since physical adsorption of particulates is a reversible phenomenon and, in part, ion-dependent. Duboise (personal communication, Samuel Monroe Duboise, The University of Texas at Austin, 1977) has monitored the movement of a genetically-distinguishable coliform organism through soil cores during cyclic applications of secondary effluent followed by distilled water. The release and subsequent movement of this organism was consistant with decreasing conductivity of the core effluents. Changes in the ionic nature of percolate waters would be expected to have the same effect in field situations.

In contrast to the assembled information on bacterial contamination, the survival of enteric viruses in natural water systems is ill-defined. This is due largely to the technical problem of monitoring their relatively low concentration in the environment and the expense incurred in carrying out investigations involving tissue culture and animals. Once again, epidemiological studies have implicated viral movement in groundwater in a number of infectious hepatitis outbreaks. Specifically, Clark and Chang (1959) list five outbreaks involving 538 cases of hepatitis in which viral-contaminated water traveled 50 to 75 feet through the soil.

A number of laboratory studies have shown that certain bacteriophages and viruses tend to adsorb to soil particles. Carlson et al (1968), reported effective adsorption of phage T2 and poliovirus to kaolinite, montmorillonite, and illite in the presence of electrolytes. In their system they also were able to elute free infectious virus from the clays. In 1968, Drewry and Eliassen found that phages T1, T2, and f2 adsorbed to nine different soil types taken from California and Arkansas. Recently, Schaub and Sagik (1975) and Moore et al (1975), have demonstrated that enteric viruses (Mengo and poliovirus 1), when associated with naturally-occurring suspended solids found in the Wichita River and in final effluent from an activated sludge treatment plant, are infectious. Further, laboratory studies by Robeck et al (1962), showed that poliovirus 1 was removed by two feet of packed sand from slow-moving water, but with increasing flow rate almost 100 percent penetration occurred. Hori et al (1970), reported that poliovirus II was able to penetrate a six-inch column of three types of Oahu soils with viral breakthrough ranging from 39 to 78 percent of that applied.

The phenomenon of adsorption as a mechanism for the retention of viruses in soil systems was demonstrated by Drewry and Eliassen (1968). The results they obtained using bacteriophage systems showed that virus adsorption followed typical Freundlich isotherms. In general, virus adsorption by soils increased with increasing ion exchange capacity, clay content, organic carbon content, and glycerol-retention capacity. The movement of poliovirus 1 (Chat) through 20 cm length, nonsterile cores taken from a sandy forest soil was monitored using simulated cycles of effluent application and rainfall (Duboise et al, 1976b). Results showed a burst of released virus detected in the core effluent as the specific conductance of the percolating water began to decrease. This pattern of movement, inversely related to specific conductance, was repeated through three cycles with 22.4% of the total virus applied being recovered in the core effluents. Additionally, these authors found the capacity of surviving virions to migrate through the soil columns during an 84-day period (during which time the natural soil moisture was maintained) was unchanged. Similar movement of poliovirus in 250 cm columns packed with calcareous sand was reported by Lance et al (1976). While most of the virus inoculum applied to the column surface in secondary effluent was adsorbed in the top 5 cm of soil, subsequent application of deionized water resulted in virus desorption and movement to a depth of 160 cm. In this study, drying for 1 day between viral application and flooding with deionized water reportedly prevented desorption (or enhanced viral inactivation).

Both laboratory and field data indicate that microbial pathogens, including viruses, can be retained in soil systems. It is equally apparent that surviving pathogens can be desorbed and moved through soil profiles given conducive ionic conditions.

Data from Operational Land Application Sites

In recent years, many municipalities practicing land application of wastewater residuals have undertaken studies of various aspects of their soil disposal systems. A brief review of selected operations may serve as a realistic starting point in assessing some of the public health aspects of land disposal.

One of the largest land disposal sites for the recycling of anaerobically digested sludges is located in Fulton County, Illinois, and operated by the Metropolitan Sanitary District of Greater Chicago. An extensive environmental monitoring system was developed in conjunction with the Illinois EPA to evaluate long-term effects of sludge disposal on land. Biological testing included fecal coliform and virus monitoring at selected surface water sites (Zenz et al, 1976). The greatest increases in fecal coliform levels due to sludge disposal could be seen in the minimal to maximal counts in field runoff water. While geometric mean values differed little, the maximal fecal coliform counts per 100 ml volumes before and after the application of digested sludge were 2.3×10^4 and 1.2×10^5, respectively. Other bacterial and viral data reported for surface streams and reservoirs showed no dramatic increases at points downstream from the disposal site when compared to upstream values. Unfortunately, insufficient information was provided in this publication to allow critical evaluation of the sensitivity of the viral recovery methods used. Additionally, no biological parameters were reported for the extensive groundwater monitoring program at the site.

A sludge disposal site operated by the East Bay Municipal Utility District is located in Solano County, California (Hyde, 1976). Anaerobically digested sludge is sprayed onto both row crop test plots and irrigated and dryland pasture at application rates ranging from 3.3 dry tons/acre to 32.3 dry tons/acre. In most instances, the applied sludges are then plowed into the upper soil layer. Three parasitic helminths (*Ascaris lumbricoides*, *Strongyloides stercoralis*, and *Hymanolepsis nana*) were found represented in the soil-sludge samples from the row crop plots. The high sludge application fields had the highest percentage of positive parasite samples. Helminth ova densities ranged from 1 to 50 per gram of soil-sludge. Significant numbers of total and fecal coliform, fecal streptococci, *Salmonella*, and *Shigella* survived for as long as seven months. Similar microbial profiles were reported for pastureland, with dryland pasture having the lowest percentage of positive parasite samples. *Streptococcus fecalis*, *Clostridium tetani*, *Clostridium perfringens*, and butyl-butyric Clostridia were found in small numbers on both irrigated and dryland pasture seven months after sludge application during the winter season. *Clostridium botulinum* was isolated at the same time from the dryland pasture.

Isolation of enteric viruses from the soils and sludges at sludge injection sites located in Butte, Montana and Boulder, Colorado was reported by Moore et al (1978). These field studies support the thesis that if viruses reach the sludge-soil matrix, extended survival can be expected, especially during extended low temperature periods.

Additional reports of pathogen isolation from wasted sludge has been published by Wellings et al (1976), in Florida. One poliovirus type 3 isolate was recovered from 500 ml of sludge after 48 hours on a spray field. Twenty-four isolates identified as Echovirus-7 were recovered from 250 grams of sludge after a 13-day period on a sludge-drying bed.

In a study using anaerobically-digested sludge to recover a forest clear-cut area in northwest Washington, Edmonds (1976) monitored the survival and movement of indigenous coliform bacteria. Fecal coliform counts in sludge applied in summer decreased from 1.1×10^5 to 3.6×10^2/gram in 204 days and were undetectable after 267 days. Coliform bacteria also moved out of the sludge layer into underlying soils. Although few bacteria moved past the first 5 cm depth, fecal coliform were recovered from ceramic lysimeters at a depth of 180 cm. Fecal coliform also were isolated from both a spring draining beneath the sludge application site and a groundwater well (52 fecal coliform/100 ml), the depth of which was not reported.

Several recent reports have considered public health aspects of land disposal of treated effluents. The Flushing Meadows Wastewater Renovation Project near Phoenix, Arizona, has been operational since 1967. Research initiated in 1974 sought to ascertain the fate of fecal coliforms, fecal streptococci, *Salmonella*, and enteric viruses (Gilbert et al, 1976). Both sewage effluent applied to the infiltration basins and renovated well water taken from depths of 6 to 9 meters were screened for these microorganisms. No viruses or *Salmonella* were detected in well samples; fecal coliform and fecal streptococci levels were diminished by 99.9%. However, difficulties attributable to the viral concentration methodology used (filter clogging and precipitate formation during reconcentration of eluates) were noted.

In contrast to these findings, Wellings et al (1975) repeatedly have isolated enteric viruses from groundwater. Monitoring a wastewater spray irrigation site at St. Petersburg, Florida, this group showed virus to have moved through 5 feet of sandy soil. Following heavy rains, viruses were isolated at this site from wells 20 to 30 feet deep. Similar isolations were made during a study of a cypress dome receiving secondary effluents. Waters from 10-foot deep monitoring wells were shown to contain virus. Two of the three positive isolations reported coincided with a period of heavy rainfall 28 days after the last application of sewage effluent.

In a study using f_2 bacteriophage as a tracer, phages were observed to travel distances in excess of 600 feet in the groundwater at a rapid infiltration site. Similar observations were made with indigenous enteroviruses (Schaub and Sorber, 1977).

POTENTIAL USE OF LAND APPLICATION SITES

The application of wastewater residuals to food crops, specifically to fruits and vegetables which may be eaten raw, raises the obvious question of possible ingestion of surviving pathogenic microorganisms. No conclusive data exist to support the uptake and subsequent translocation of pathogens from contaminated soil into non-traumatized edible plant tissues. However, various studies have shown survival times ranging from hours to months for microbial pathogens applied to the surface of fruits and leafy vegetables (Dunlop, 1968). Rudolfs et al (1951a, b, c) in a series of papers cited 7-day survivals for *Salmonella* and *Shigella* on tomatoes, 3 days of dry weather survival for *E. histolytica* cysts on lettuce and tomatoes, and 35 days for immature *Ascaris* ova.

Bagdasaryan (1964) reported survival of enteroviruses on artificially contaminated tomatoes and radishes over a period of two weeks under household storage conditions. Larkin et al (1976) added poliovirus I to wasted sludge and secondary effluent used to spray irrigate a series a test plots planted with lettuce and radishes. Test plots were exposed to prevailing environmental conditions including soil-surface temperatures reaching 45C and rainfall. As expected, greater viral numbers were observed on the sludge-irrigated plants due to retention of particulates. A two $\log_{10}$ loss of detectable virus during the first 5 to 6 days was reported for the first study from August through October, 1973. In the 1974 study, conducted from June through August, heavy rains fell immediately after the last spray irrigation; most of the poliovirus applied apparently was flushed from the plants and subsequently detected in runoff at a concentration of 500 pfu/ml.

Common sense suggests that non-agricultural uses of treatment plant residuals could gain wide acceptance. Enhancement of parks and forests and reclamation of marginal and damaged soils are apparent appropriate applications. There is some question as to whether such sites ought to be used for the growth of human food crops eaten raw. Forage and pasture crops may not need the same degree of caution.

SECTION 5

OBJECTIVES

The overall objective of this study was to evaluate the survival and transport of enteric viruses at land application sites for wastewater following conventional treatment. Specific, detailed objectives were:

(1) To determine the survival of enteroviruses distributed to the soil following wastewater irrigation.

(2) To follow the movement of indigenous enteroviruses and bacteriophages specific to *Escherichia coli* through the unsaturated soil zone at a wastewater irrigation site, using lysimeters placed at depths to 4.5 feet at several locations within that site.

(3) To ascertain possible movement of indigenous enteroviruses and bacteriophages to the groundwater beneath a wastewater land application site by regular sampling of monitoring wells installed at various locations on the site.

(4) To conduct a mass balance of indigenous enteroviruses entering the site by wastewater irrigation and remaining in the soils on the site or leaving the site through transport in the groundwater or by surface run-off.

(5) To determine the role of pond sediments as a potential virus reservoir in irrigation with wastewaters.

(6) To initiate the study of the presence in wastewater of specific bacterial pathogens at the irrigation site under study.

SECTION 6

DESCRIPTION OF STUDY SITES

Several wastewater land application sites were considered for inclusion in this study. The selection of study sites was based on the following criteria:

1. The availability of at least 1.0 mgd of wastewater, primarily domestic in origin, which is subjected to conventional wastewater treatment;
2. The existence of a land application operation of sufficient size so that research could be conducted on an uninterrupted basis;
3. The willingness of municipal officials to cooperate in the research project; and,
4. The proximity of the site to The University of Texas at San Antonio to facilitate sampling and analysis.

Although several potential sites were considered, most were eliminated as they did not satisfy at least two of the first three stated criteria. Through field investigations and preliminary analysis, the number of sites selected for study was reduced to two. A detailed description of these sites follows.

KERRVILLE, TEXAS

Kerrville's wastewater (an average of approximately 1.4 mgd) is generated from a population of approximately 15,500 and enters the treatment plant where it is divided between two essentially separate treatment systems (see Figure 1). The first 800,000 gpd is diverted to a new oxidation ditch system and the remainder is divided equally between the oxidation ditch and a trickling filter system. Each system has its own clarifiers. From the final clarifiers all wastewater is discharged to an irrigation pond with a storage capacity of approximately 75 acre-ft. The oxidation ditch became completely operational in 1977. Although completed in early 1976, initial problems (including sludge handling) precluded its normal operation until early in 1977.

An aerial photograph presented as Figure 2 shows the location of both the treatment plant and the land application site, including the irrigation and tailwater ponds. The irrigation system is of the fixed

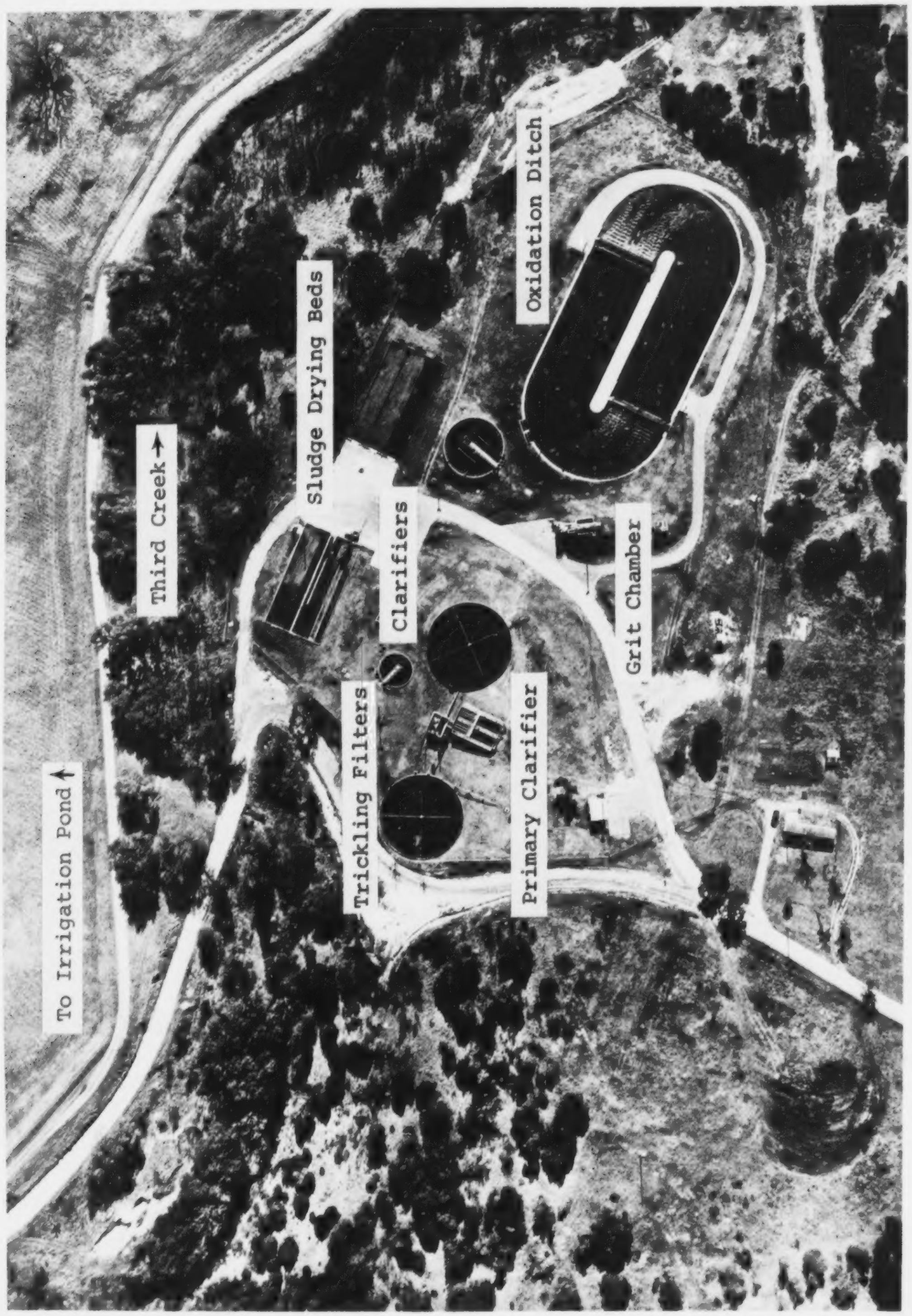

To Irrigation Pond
Third Creek
Sludge Drying Beds
Oxidation Ditch
Clarifiers
Trickling Filters
Primary Clarifier
Grit Chamber

FIGURE 1. Wastewater Treatment Facilities - Kerrville Site

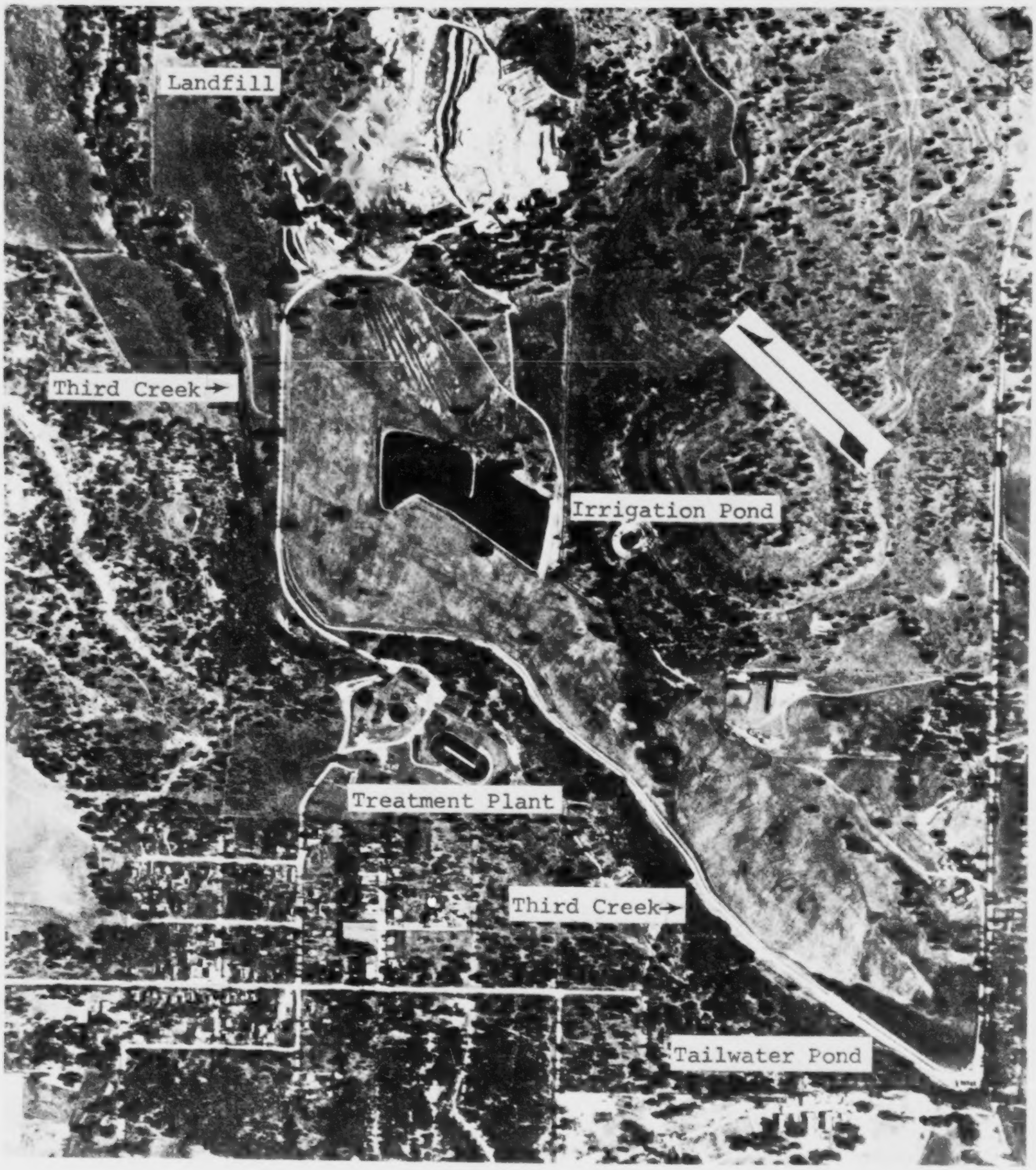

Landfill
Third Creek→
Irrigation Pond
Treatment Plant
Third Creek→
Tailwater Pond

FIGURE 2. Aerial View of the Kerrville Site.

type covering an area of approximately 135 acres. Laterals (4 inches and 6 inches) are trenched to 3.0 ft. and spaced at a nominal 200 ft. along a 12-inch irrigation main fed by the system pumps. Impact sprinkler heads (Buckner No. 250G) are fixed on 18-inch risers above the ground and are rated at 140 gm at 80 pse. They are spaced at 100 ft. intervals staggered at adjacent laterals. The sprinkler system ws designed and installed as a fully automated system. Current operaton (1.4 mgd) results in a mean application rate of 3 inches/week at a 90% operating factor. Design flow (2.25 mgd), when reached will result in a mean application rate of 4.8 inches/week.

Soils at this site vary in type from loams to clays over dstinctly classified segments of the site (see Figure 3). Prior to the installation of the present system (1976) approximately 50 acres were never under irrigation, while other portions of the site have been flood irrigated since 1930. Other sections have been flood irrigated since 1950 and some areas were irrigated between 1930 and 1960 only. Consequently, a variety of soil types and prior irrigation situations exist at one site. Coastal Bermuda grass is the present crop on the site and it is harvested 2 to 3 times each year. Further, the site is used as pasture for a limited number of cattle.

Site Geology

The study site is located in the Edwards Plateau physiographic province. The plateau region is strongly dissected by stream erosion yielding a rugged topography refered to as the Texas Hill Country.

The streams in the area have eroded headward to form narrow valleys with steep walls of limestone and linestone marls of the Upper Glen Rose Formation. Outcrops of carbonate strata are present but are limited to the walls of the valley. Third Creek is incised some 160-170 feet below the interstream divides. Valley slopes are concave with slope breaks formed by resistant limestone beds within the Upper Glan Rose.

Third Creek is a perennial stram and is spring fed in the upper and middle reaches. Drainage to the creek is well integrated with no observed swales or depressions. Alluvial materials in the valley are mixtures of flood plain terrace and alluvial fan deposits. The deep alluvial soils in the valley are developed on these alluvial materials.

The Upper Glen Rose Formation is composed of alternating beds of limestone, dolomite and marly limestone. In the study area the Upper Glen Rose is approximately 350 feet thick. Erosion of the Glen Rose and the overlying Edwards Formation on the interstream divides has produced the alluvial materials in the Third Creek Valley.

Soil of the Study Site

The Kerrville site is located in an area of thick alluvial soil and thin upland soils. Soil series as mapped by the U.S. Department of Agriculture Soil Conservation Service are shown in Figure 3 and include the Brackett, Frio, Krumm, Orif, Denton, Doss and Lewisville Series.

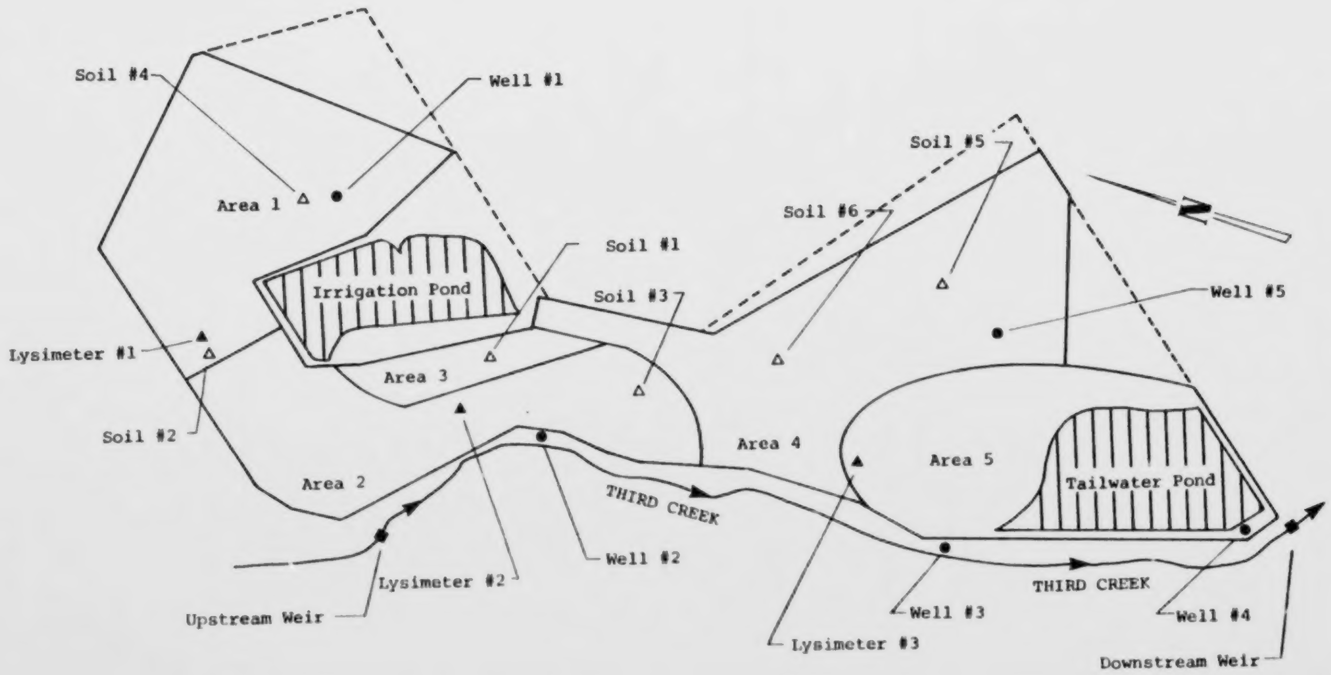

Soil #4
Well #1
Area 1
Irrigation Pond
Soil #1
Soil #3
Lysimeter #1
Area 3
Soil #2
Area 2
Upstream Weir
Lysimeter #2
Well #2
THIRD CREEK
Soil #6
Soil #5
N
Well #5
Area 4
Area 5
Tailwater Pond
Lysimeter #3
Well #3
THIRD CREEK
Well #4
Downstream Weir

FIGURE 3. Location of Wells, Lysimeters, Soil Sampling Points and Weirs, Kerrville Site

LEGEND FOR FIGURE 3

Area	Prior Irrigation*	Type Area	Soil Classification and Description **
1	None	a. Burn and cover garbage area b. Sanitary landfill area c. Cropland	IIIe-5. Denton Clay, shallow, 1-5% slope Shallow, moderate to highly erodible, gently sloping to sloping clays or silty clays 10-20 inches deep. and I-1. Lewisville Clay Loam, 0-1% slope Deep, nearly level, dark grayish brown and pale brown silty clay loam and clay soils which take water readily and have adequate water and fertility storage capacity.
2	Flood Irrigated since 1930	Cropland	IIc-1. Lewisville Clay Loam, 0-1% slope Catalpa Clay Loam, high bottom, 0-1% slope Catalpa fine sandy loam, 0-1% slope Deep nearly level soils with moderate permeable subsoils.
3.	Flood Irrigated between 1930 and 1960	Cropland	IIc-1. Lewisville Clay Loam, 0-1% slope Catalpa Clay Loam, high bottom, 0-1% slope Catalpa fine sandy loam, 0-1% slope Deep nearly level soils with moderately permeable subsoils.

LEGEND FOR FIGURE 3 (Continued)

4	None	Pasture	IIIe-5. Denton Clay, shallow, 1-5% slope Shallow, moderate to highly erodible, gently sloping to sloping clays or silty clays 10-20 inches deep and IIIe-3. Krum Clay, 3-5% slope Denton Clay, 3-5% slope Deep, highly erodible, sloping clay and silty clay loam soils with moderately permeable subsoils.
5	Flood Irrigated since 1950	Cropland	IIc-1. Lewisville Clay Loam, 0-1% slope Catalpa Clay Loam, high bottom, 0-1% slope Catalpa fine sandy loam, 0-1% slope Deep nearly level soils with moderately permeable subsoils.

---

* Prior to installation/operation of the present sprinkler installation system (February, 1976)

** U.S. Department of Agriculture, Soil Conservation Service, Kerrville, Texas

The Orif Series is confined to a narrow flood plain adjacent to Third Creek. The irrigated area does not include this soil series. In addition, the Krumm Series occupies a very small portion of the upland area and is not included in the irrigated area.

Lewisville Series (13A1)--

The Lewisville Series is a member of the fine-silty mixed, thermic family of Typic Calclustolls. A representative profile is given below (1):

Ap	0-6" -- Dark grayish brown (10YR 4/2) silty clay; very dark grayish brown (10YR 3/2) moist; moderate very fine subangular blocky and granular structure; hard, friable; contains a few strongly cemented $CaCO_3$ concretions; calcareous; abrupt smooth boundary. (0 to 7 inches thick)
A1	6-16" -- Dark grayish brown (10YR 4/2) silty clay, very dark grayish brown (10YR 3/2) moist; moderate fine subangular blocky structure; hard, firm; few root channels; common strongly cemented $CaCO_3$ concretions about 2 to 5 mm in diameter; calcareous; gradual smooth boundary. (7 to 15 inches thick)
B21ca	16-34" -- Grayish brown (10YR 5/2) silty clay, dark grayish brown (10YR 4/2) moist; moderate fine subangular blocky structure; very hard, firm; common strongly cemented $CaCO_3$ concretions 2 to 5 mm in diameter; a few threads of soft $CaCO_3$; calcareous; gradual smooth boundary. (13 to 30 inches thick)
B22ca	34-62" -- Pale brown (10YR 6/3) silty clay; brown (10YR 5/3) moist; weak subangular blocky structure; hard, firm; common soft masses of segregated $CaCO_3$, few small, strongly cemented $CaCO_3$ concretions; calcareous.

The Lewisville Series is typically well drained, moderately permeable, calcareous soils. The soils occupy level to sloping terrace deposits, with a slope range of 0 to 10%. Soil permeability is moderate, ranging from 0.6 - 2.0 in/hr. The soil is well drained with slow to medium runoff.

Frio Series (26A1)--

The Frio Series is a member of the fine, mixed thermic family of Cumulic Haplustolls. A representative profile is given below (1):

A11	0-22" -- Very dark grayish brown (10YR 3/2) silty clay, very dark brown (10YR 2/2) moist; strong fine granular structure in the upper 4 inches and moderate fine subangular blocky structure below; hard, firm, sticky, plastic, COLE is .065; many fine tree and grass roots;

few snail shells; 22 percent calcium carbonate equivalent; few films and threads of $CaCO_3$ visible in lower part when dry; calcareous, moderately alkaline; diffuse smooth boundary. (12 to 28 inches thick)

A12 22-40" -- Very dark grayish brown (10YR 3/2) silty clay, very dark brown (10YR 2/2) moist; moderate fine and medium subangular blocky structure; hard, firm, sticky, plastic; COLE is .060; few fine tree roots; 27 percent calcium carbonate equivalent; many threads and films of $CaCO_3$ visible when dry; calcareous; moderately alkaline; gradual smooth boundary. (10 to 30 inches thick)

Cca 40-62" -- Dark grayish brown (10YR 4/2) silty clay, very dark grayish brown (10YR 3/2) moist; massive; hard, firm, sticky, plastic; few thin strata of very dark grayish brown silty clay; a few thin bedding planes in lower part; 29 percent calcium carbonate equivalent; many films and threads of $CaCO_3$ and a few soft powdery masses of $CaCO_3$; calcareous; moderately alkaline.

The Frio Series consists of well-drained, level soils of the bottom lands. The soil is formed from calcareous alluvial materials. The soil is well-drained with slow runoff and has a moderately slow permeability, ranging from 0.2-0.6 in/hr.

Denton Series

The Denton Series is a member of the fine, montmorillonitic, thermic family of Vertic Calclustolls. A representative profile is given below (1):

Ap 0-6" -- Dark grayish brown (10YR 4/2) silty clay, very dark grayish brown (10YR 3/2) moist; moderate medium and fine granular and subangular blocky structure; hard, firm, sticky and plastic; many roots; few fine fragments of limestone; calcareous; moderately alkaline; clear smooth boundary. (4 to 8 inches thick)

A11 6-14" -- Brown (7.5YR 4/2) silty clay, dark brown (7.5YR 3/2) moist; moderate medium and fine subangular blocky structure; very hard, firm, sticky and plastic; many roots; common very fine pores; few partially sealed cracks filled with material from above; few fine fragments of limestone; calcareous; moderately alkaline; gradual wavy boundary. (5 to 15 inches thick)

A12 14-26" -- Brown (7.5YR 4/3) silty clay, dark brown (7.5YR 3/3) moist; moderate medium and fine angular and subangular blocky structure; very hard, firm, sticky and plastic; many roots; common very fine pores; common shiny pressure faces; vertical cracks filled with dark grayish brown (10YR 4/2) silty clay; few fine fragments of limestone; calcareous; moderately alkaline; gradual wavy boundary. (7 to 16 inches thick)

Bca 26-34" -- Brown (7.5YR 5/4) silty clay, brown (7.5YR 4/4) moist; moderate medium and fine angular and subangular blocky structure; hard, firm, sticky and plastic; few roots; few dark streaks; common limestone fragments; few fine weakly cemented $CaCO_3$ concretions; calcareous; moderately alkaline; abrupt irregular boundary. (0 to 19 inches thick)

Cca 34-38" -- Mixture of about 80 percent flaggy limestone fragments, some of which can be cut with a spade and 20 percent brown (7.5YR 5/4) silty clay; massive soil is hard, firm, sticky and plastic; few roots; limestone flags are up to 2 inches thick and 12 inches across the long axis; common soft masses of $CaCO_3$; calcareous; moderately alkaline; abrupt irregular boundary. (0 to 20 inches thick)

R 38-60" -- Fractured limestone that cannot be cut with a spade, interbedded with calcareous clayey marl.

The Denton Series occurs on level to very gently sloping uplands. The soil is formed from a mantle of marly materials over weakly cemented to fractured limestones and marly limestones. The soil is typically well-drained with rapid runoff. Soil permeability is slow-ranging from 0.06 - 0.2 in/hr.

Doss Series

The Doss Series is a member of the loamy, carbonatic, thermic, shallow family of Typic Calciustolls. A representative profile is given below (1):

A1 0-8" -- Dark grayish brown (10YR 4/2) silty clay, very dark grayish brown (10YR 3/2) moist; moderate fine and medium subangular blocky structure; very hard, very firm, very sticky and plastic; many fine and medium grass roots; common fine pores; common very fine soft bodies of $CaCO_3$; about 3 percent weakly cemented fragments of $CaCO_3$ about 1/4-inch across the long axis; calcareous; moderately alkaline; clear smooth boundary. (7 to 12 inches thick)

B2ca 8-19" -- Brown (10YR 5/3) silty clay, dark brown (10YR 4/3) moist; moderate fine and medium subangular blocky structure; very hard, very firm, very sticky and plastic;

common fine and few $CaCO_3$; few angular fragments of weakly cemented limestone up to 1/4-inch across the long axis; calcareous, moderately alkaline; clear smooth boundary. (4 to 13 inches thick)

Cca 19-48" -- Very pale brown (10YR 8/4) weakly cemented marlaceous limestone interbedded with silty clay, very pale brown (10YR 7/4) moist; platy in the upper 3 inches with hardness of 2.0 on Mohs scale, massive below and hardness of about 1 on Mohs scale; many veins and bodies of $CaCO_3$; calcareous, moderately alkaline.

The Doss Series is typically a shallow, upland soil formed on marly limestone and weakly cemented limestone. The soils occupy nearly level to moderate slopes. The soils are well-drained with medium runoff. Soil permeability is moderately slow-ranging from 0.2 - 0.6 in/hr.

Brackett Series (2EG)

The Brackett Series is a member of the loamy, carbonatic, thermic, shallow family of Typic *Ustrochrepts*. A representative profile is given below (1):

A1 0-6" -- Light brownish gray (2.5YR 6/2) loam, grayish brown (2.5Y 5/2) moist; moderate fine and very fine granular and subangular blocky structure; hard, firm; many grass roots; many worm casts of lighter colored material from horizon below; about 3 percent fragments of limestone mostly 5 to 15 mm in diameter; the bulk of these are on the surface as a "pavement"; $CaCO_3$ equivalent is about 55 percent; calcareous; moderately alkaline; clear wavy boundary. (3 to 12 inches thick)

B2 6-16" -- Pale yellow (2.5Y 8/4) loam, pale yellow (2.5Y 7/4) moist; moderate very fine subangular blocky structure; hard, friable; many roots; about 5 percent, by volume of subrounded weakly and strongly fragments of limestone, mostly 2 to 15 mm in diameter; common tongues of darker soil from layer above in old root channels or cracks; a few soft bodies of $CaCO_3$; $CaCO_3$ equivalent is about 65 percent; calcareous; moderately alkaline; clear boundary. (4 to 16 inches thick)

C 16-50" -- Thinly interbedded weakly and strongly cemented platy limestone and pale yellow calcareous clay loam, cleavage planes of rock structure are evident in both the limestone and in the clay loam; few roots in the upper part in vertical crevices and between the horizontal plates of the limestone.

The Brackett Series occurs on the uplands and is formed from interbedded limestone and marly limestone. The soils are well-drained with rapid runoff. Soil permeability is moderately slow, 0.2 - 0.6 in/hr.

UVALDE, TEXAS

The treatment facility operating at the Uvalde site is shown in Figure 4. Approximately 3.0 mgd of domestic wastewater generated from a population of approximately 9,000 is treated by preaeration, primary settling and trickling filtration prior to discharge to a series of six holding ponds having a maximal capacity of about 96 acre-ft. Treated wastewater then flows by gravity to another pond adjacent to Cook's Slough from which it can be pumped to irrigate approximately 41 acres of cropland and 22 acres of pasture land. Two additional ponds collect some of the irrigation run-off prior to entry to Cook's Slough.

In practice, the municipality operates the land application project by periodically irrigating the cropland, although only part of the wastewater is used. For study purposes, selected areas can be isolated for controlled application. At present, the irrigation operation is not highly structured.

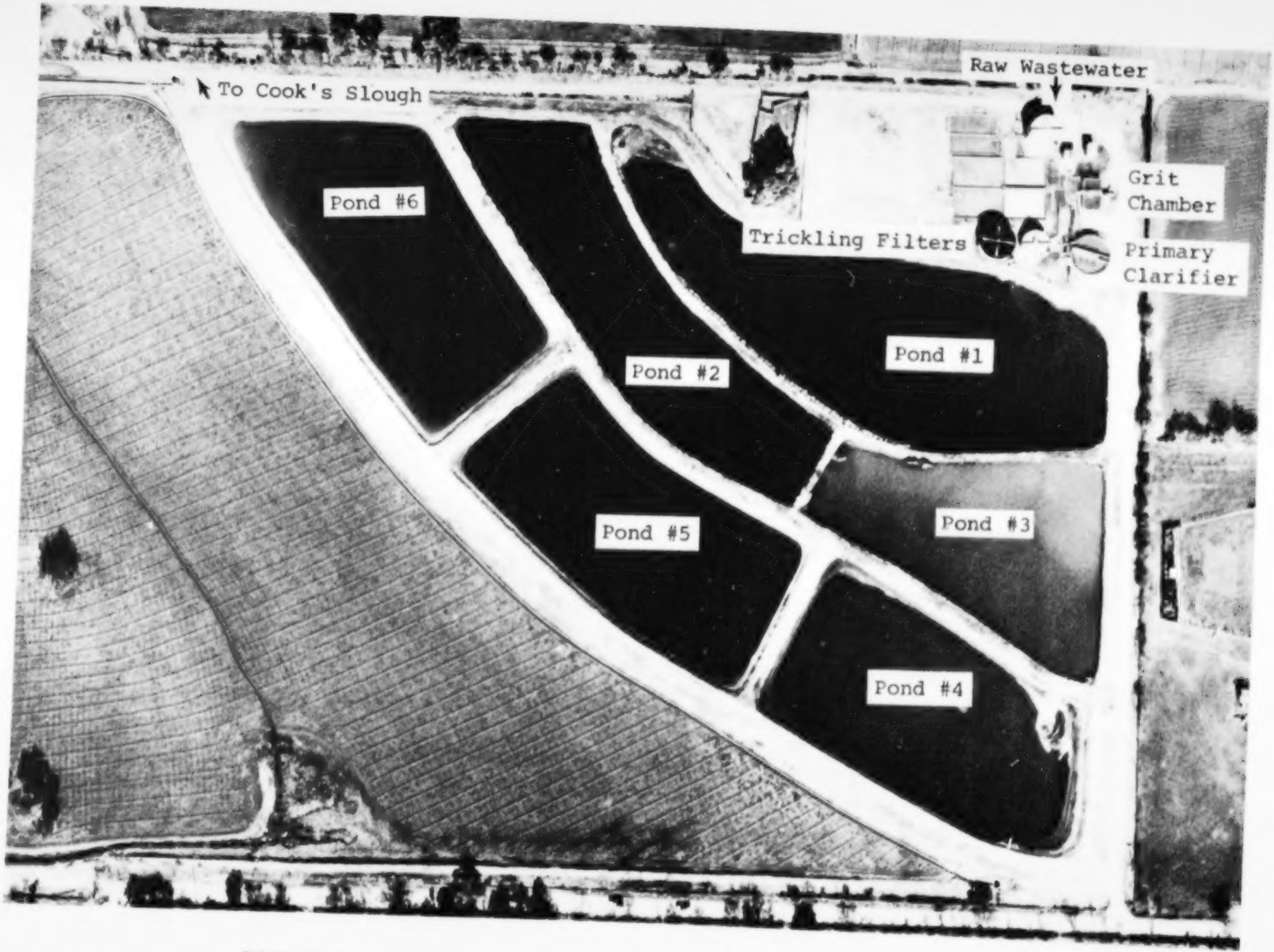

Raw Wastewater
To Cook's Slough
Grit Chamber
Trickling Filters
Primary Clarifier
Pond #6
Pond #1
Pond #2
Pond #3
Pond #5
Pond #4

FIGURE 4. Wastewater Treatment Facilities - Uvalde Site

SECTION 7

METHODS AND MATERIALS

LYSIMETERS

Although relatively simple, the lysimeters designed for use in this study appear to serve adequately for sampling water from the percolation zone when saturated. They are constructed of a single piece of 3/4-inch schedule 40 PVC pipe 11 inches long. The PVC pipe is capped at one end and a threaded hose fitting is attached to the other end. Twelve 60-gauge diameter holes are drilled into the lower portion of the pipe. The pipe is mounted in a shallow polyethylene pan 7 inches wide X 10 inches long X 5 inches deep with the hose fitting extended through one end of the pan. The pan is filled with washed gravel graded from 1/4-inch diameter surrounding the pipe to fine washed sand at the edges and top of the pan. An evacuation hose, 1/4-inch rigid polyethylene, is attached to the hose fitting and brought to the surface. Details of lysimeter construction can be found in Figure 5.

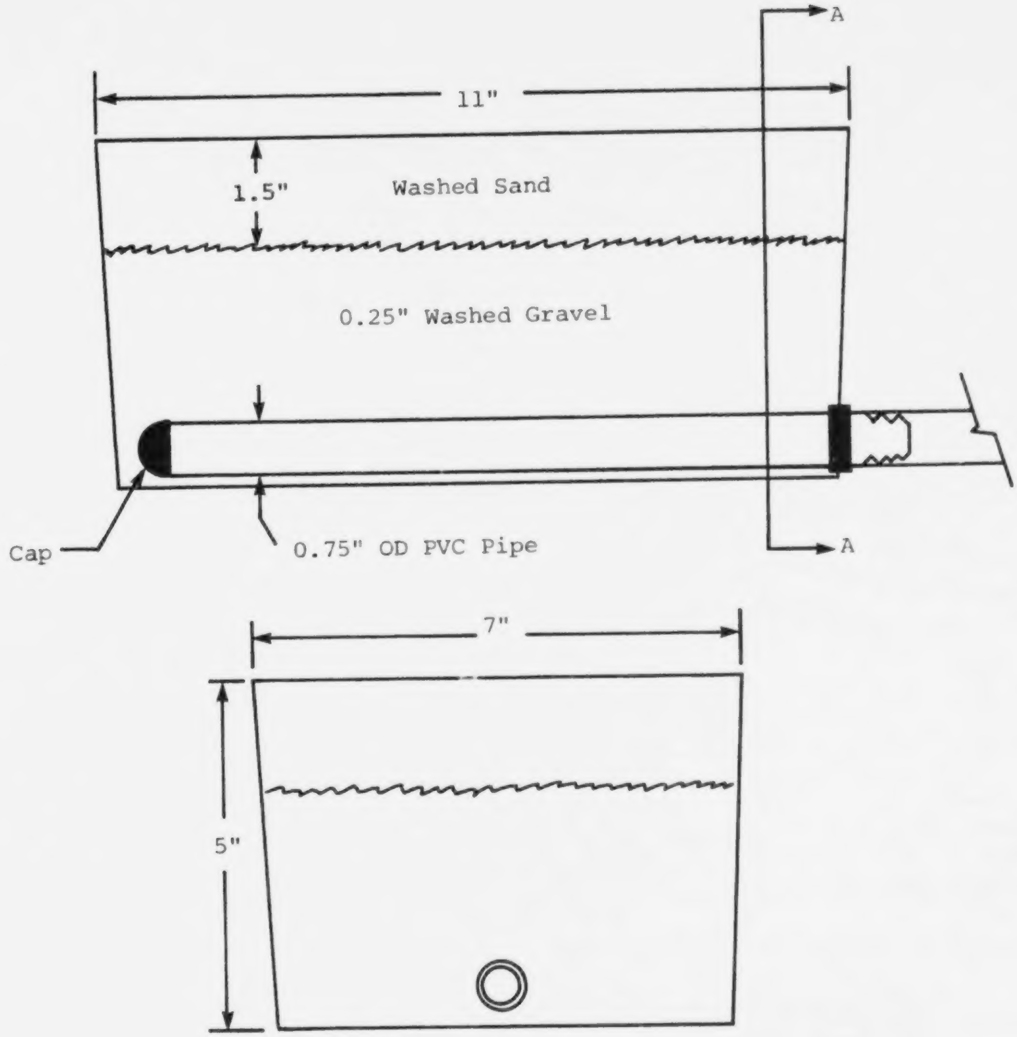
Three lysimeter locations were selected at the Kerrville site (see Figure 3). A trench approximately 2 feet wide and 6 feet deep was dug at each location. The lysimeters were installed at depths of 1.5, 3.0 and 4.5 feet beneath the surface. By burrowing into the side walls of the trenches, horizontal staggering between lysimeters was maintained. In addition, a soil moisture probe was installed adjacent to each lysimeter. The trenches were backfilled following installation of lysimeters and soil moisture probes.

MONITORING WELLS

As previously discussed, Third Creek (Kerrville site) is located in a valley formed on the Upper Glen Rose Formation. Using a characteristic cross section of stream valleys in the Edwards Plateau region, a generalized geologic cross-section is given in Figure 6.

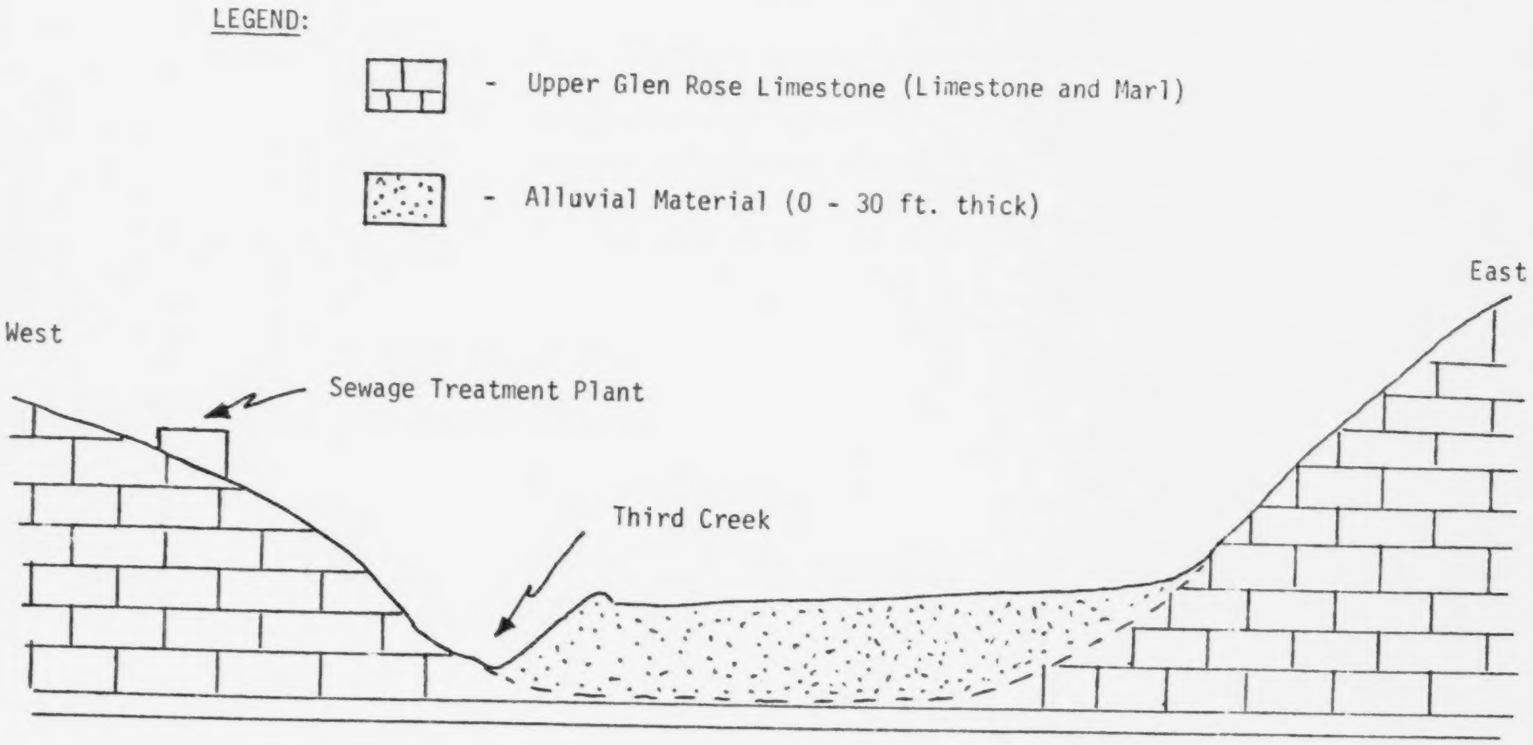
Groundwater movement in the irrigated field area should follow the topography of the area -- that is, down-slope towards Third Creek and, once within the thick alluvial sequence, down the stream valley. Numerous small, shallow water wells have been completed in these alluvial materials in Kerr County. However, all major, large water wells near this site are more than 600 feet in depth and have been completed in the Sligo and Hosston Formations.

Well #1 was located to permit groundwater sampling in an area of


A
11"
1.5"
Washed Sand
0.25" Washed Gravel
Cap
0.75" OD PVC Pipe
A
7"
5"

Section A-A

FIGURE 5. Details of Polypropylene Pan Lysimeter.



LEGEND:
- Upper Glen Rose Limestone (Limestone and Marl)
- Alluvial Material (0 - 30 ft. thick)
West
East
Sewage Treatment Plant
Third Creek

FIGURE 6. Generalized Geology at the Kerrville Irrigation Site

shallow bedrock. Samples taken at this location should indicate any movement of the effluent down into the limestone bedrock and subsequent lateral movement. Also, this location would allow the sampling of any leachate from the up-slope solid waste disposal site. If no anomalous water quality concentrations were encountered at this site, it would be reasonable to assume that there were no complicating factors as a result of leachate.

Two sampling wells (#2 and #3) were located along the western border of the irrigation field and designed to intercept the shallow groundwater in the alluvial materials. Well #2 was placed immediately downslope from the irrigation pond. If there was continuous downward percolation and lateral movement of effluent from the pond, this well location was in an excellent position to sample the resulting groundwater quality. Well #3 was designed to sample groundwater quality in an area that would have effluent application at intermittent intervals rather than continuously as at Well #2. Again, the well sampled water from the shallow groundwater in the alluvial materials.

Well #4 was located down valley from the tailwater pond. Groundwater quality in this location should reflect the contribution from the spray irrigated area, downward percolation of run-off, and down valley movement of groundwater from areas upstream from the irrigation site.

The fifth well (Well #5), as with Well #1, sampled groundwater in an area of relatively thin soil cover, no underlying alluvial materials with limestone and marl bedrock. The downward percolation and lateral movement of effluent and the resultant water quality could be monitored without the potential complications of the solid waste leachate at Well #1.

Special care was taken in the construction of the monitoring wells to preclude direct contamination by irrigation wastewater (see below). For specific well locations, see Figure 3.

Well Installation

The monitoring wells were drilled with an 8-inch diameter bit to total depth. Natural mud and water were used as drilling fluids in Wells #1 and #4 to prevent contamination of the surrounding formations by exotic drilling muds. Wells #2, #3 and #5 were drilled with natural fluids and a commercial biodegradable detergent. The detergent was used to increase the viscosity and hence the cutting return capability of the drilling fluid. Wells completed with the soaping agent showed no measurable detergent prior to the initiation of routine sampling.

Each monitoring well was completed with a 5-foot long PVC-covered well screen equipped with a check valve in the base. Well screen opening diameters were 0.01-inch. Production casing was 5-inch schedule 40 PVC pipe. In order to prevent any contamination from surface run-off moving down the annular space, each well was completed with a PVC sheet wrap packer set below the base of the soil zone and cemented back to the surface (see Figure 7). Each well was tested for packer leaks by utilizing pH measurements to detect the presence of cement in the well water.

Sampling of the monitoring wells was by submersible centrifugal pumps mounted at the well screen. Each well was equipped with a 1/3 horsepower, 115 VAC pump rated at 12.0 gpm at a depth of 40 ft. with a discharge pressure of 20 psi.

Well Drilling Sample Study

Samples were collected from drilling operations at each monitoring well site at 1 to 2-foot intervals. The samples were examined in the laboratory for mineralogical content, porosity and textural features such as grain size, rounding, sorting and degree of cementation. A generalized well log for each well can be found in Figure 8.

Well Development

After the observation wells were drilled and completed, each well was tested for yield and chemical quality. Difficulties were encountered with Wells #2 and #5. Both wells had a very low yield and water from Well #5 had an abnormally high pH (pH > 12). The high pH was indicative of a cement leak past the packer. In order to improve the yield and to remove any cement from the well annulus below the packer, the well was acidized with 150 gallons of 30% hydrochloric acid (HCl). After one hour, the well was flushed with fresh water, bailed and pumped. After development, the pH in the well dropped to a normal range of groundwater in this region and the yield was substantially improved.

Well #2 was acidized solely to improve the yield, utilizing 150 gallons of 30% HCl. The yield of the well failed to improve. It was then surged and air shocked using both high pressure water and air. Unfortunately, despite this extensive development, the yield of Well #2 remained minimal.

Direction of Groundwater Flow

The elevation of each monitoring well was determined. Water levels in each monitoring well were measured over a sufficient period of time to determine the configuration of the piezometric surface at the Kerrville site. The relationship of groundwater flow direction and the movement of various wastewater contaminants in the subsurface can be determined through the construction of groundwater contour maps.

SURFACE WATER MEASUREMENTS

Precipitation and Irrigation

Initially, gauges to measure rainfall and irrigation were constructed of modified Buchner funnels (9 cm diameter) affixed to either one or 5 liter plastic bottles. These gauges were mounted 1.5 ft (irrigation

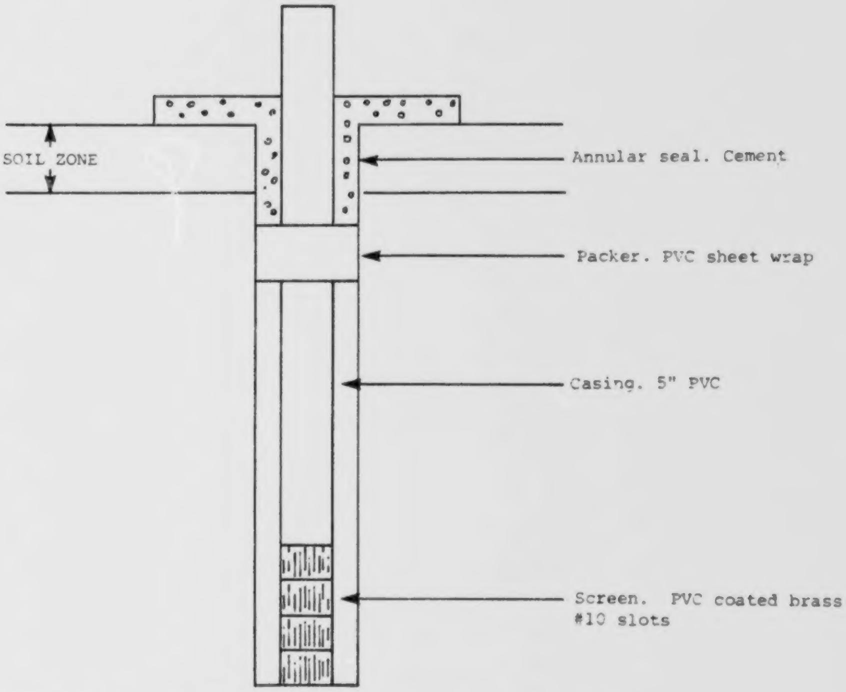

SOIL ZONE
Annular seal. Cement
Packer. PVC sheet wrap
Casing. 5" PVC
Screen. PVC coated brass
#10 slots

FIGURE 7. Monitoring Well Completion Design

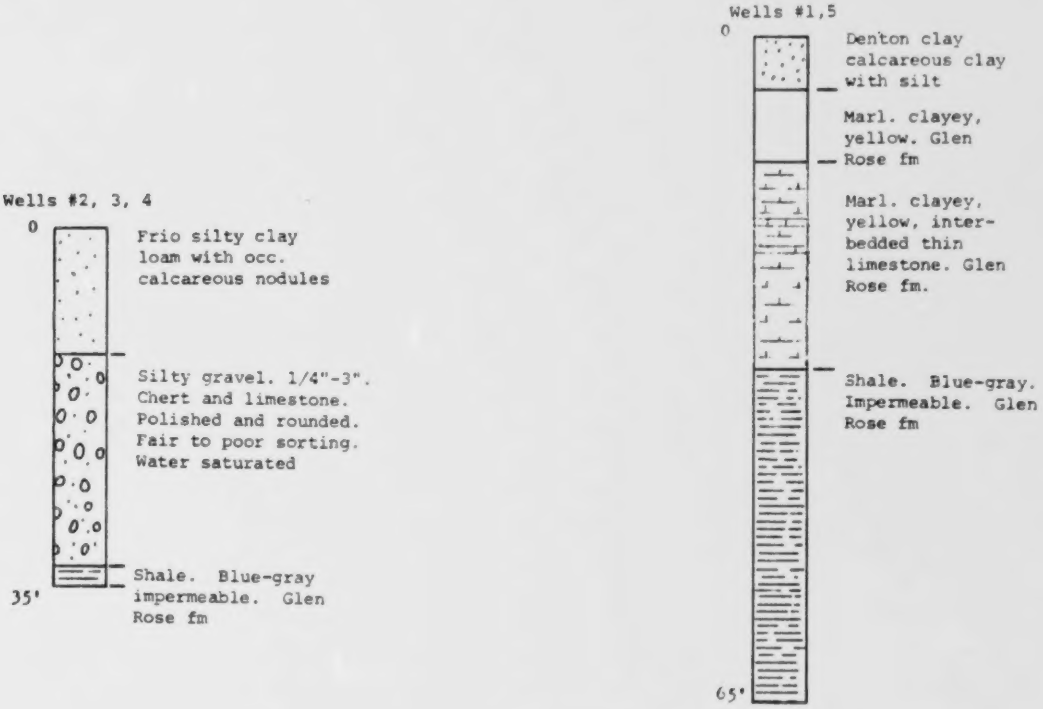

Wells #2, 3, 4
0
Frio silty clay
loam with occ.
calcareous nodules
Silty gravel. 1/4"-3".
Chert and limestone.
Polished and rounded.
Fair to poor sorting.
Water saturated
Shale. Blue-gray
impermeable. Glen
Rose fm
35'
Wells #1,5
0
Denton clay
calcareous clay
with silt
Marl. clayey,
yellow. Glen
Rose fm
Marl. clayey,
yellow, inter-
bedded thin
limestone. Glen
Rose fm.
Shale. Blue-gray.
Impermeable. Glen
Rose fm
65'

FIGURE 8. Generalized Well Log for Monitoring Wells at Kerrville Site

sampling) to five feet (rainfall sampling) above the field surface. Volumes in the collection vessels were recorded at regular intervals (generally one week) to allow cumulative measurement of precipitation over a period of time. The gauges worked very effectively for the measurement of irrigation but proved inadequate for rainfall. Evaporative loss from the one liter bottles and damage caused by roosting birds necessitated an alternate approach.

A remote recording rain gauge (Weather Measure Corp. Model 5501-1) was installed on the site late in the study. The gauge has a 20 cm diameter orifice with a tipping bucket mechanism calibrated to tip after each 0.01 inch of rainfall. The rain gauge was coupled electronically to a self-contained, battery-operated event recorder (Weather Measure Corp. P520) equipped with a seven-day chart recorder. The rain gauge was secured to a concrete pad within a fenced enclosure, and the recorder was installed inside an existing building approximately 45 feet away.

Pond Volumes

The ponds were surveyed and measured for depth utilizing standard surveying techniques. The perimeter of each pond was measured with steel tape and compass at a known water level. Approximate pond bottom contours were measured utilizing a rigid depth staff from a boat. Depth measurement locations were recorded by triangulation from fixed points on shore.

Pond volumes were calculated from these measurements and pond level readings regularly taken from calibrated staves fixed on piers in both ponds.

Creek Flow

Flow in Third Creek at the Kerrville site was established using 90° V-notch weirs and level recorders. Two 90° V-Notch weirs were installed on Third Creek as indicated on Figure 3. Continuous level recorders were installed in still-wells behind each of the weirs to record fluctuations in the flow of the creek.

The upstream weir was installed in a concrete double-piped culvert. A circular plywood plug was placed in one of the concrete pipes and caulked in place. The other pipe was fitted with a notched circular piece of plywood onto which was mounted the 90° V-notch weir. A staff was fixed to the solid plywood plug. The still-well and recorder were mounted on a sandbag supported wooden platform.

The downstream recording device consisted of a 4 ft x 8 ft plywood dam onto which was mounted the 90° V-notch weir. The plywood dam was placed in a trench dug across the stream bed and then sandbagged in place providing structural support for the dam. The still-well and recorder were placed about 8 feet upstream from the weir.

SAMPLING

Water

Sampling at the Kerrville and Uvalde plant sites was planned initially to produce results that would be average in nature and would not represent extremes. This is particularly important when dealing with wastewater treatment facilities which commonly are subjected to extreme loading variations. Thus, a combination of flow composite, time composite and grab sampling techniques were employed. Table 9 provides a summary of the sampling methods employed.

Flow Composite Samples--

Twenty-four hour flow composite samples were constructed by collecting fixed volumes at 4-hour intervals and recording total flow for the sum of the 2 hours before and the 2 hours after the sampling time. At the end of the 24-hour period, the individual samples were composited into a larger sample as a direct proportion to the flow which occurred during the sampling period. For example, samples could be collected at 12, 4, 8pm and 12, 4 and 8am. Total flow was determined for the 24-hour period as well as for the periods 10am - 2pm, 2-6pm, 6-10pm and so forth. A fixed volume of composite sample (say 10 liters) was made up from the respective individual samples as a percentage of the total flow which occurred during the sampling period (i.e., if 20% of the total daily flow occurred between 10am and 2pm, the 10-liter composite sample would contain 2.0 liters of sample collected at 12pm). Experience has shown that this method provides a representative daily composite sample.

Time Composite Samples--

For those sampling points which were expected to show dampened pollutant variation and minimal flow variation throughout a 24-hour period, a time composite sample was used. This procedure usually involved collecting samples at fixed time intervals (say every 8 hours). When all samples were collected, they were formed into a composite sample by taking equal volumes.

Grab Samples--

Collection of a single grab sample was used when there was little chance of significant flow or pollutant characteristic changes throughout any 24-hour period.

Soils

Soils were collected from six sampling locations at the Kerrville site and from four sampling locations at the Uvalde site. Soil to a depth of approximately three centimeters from the surface was collected and placed in appropriate containers, depending on the analysis to be performed.

TABLE 9. Sampling Points and Sampling Method

	Sampling Method		
Sampling Point Identification	24-Hour Composite		
	Flow	Time	Grab
Kerrville			
Influent (Raw Wastewater)	X		
Oxidation Ditch Secondary Clarifier Effluent	X		
Trickling Filter Secondary Clarifier Effluent	X		
Irrigation Pond			X
Tailwater Pond			X
Third Creek, Upstream of Site		X	X
Third Creek, Downstream of Site		X	X
Lysimeters			X
Monitoring Wells			X
Soils			X
Uvalde			
Influent (Raw Wastewater)	X		
Trickling Filter Secondary Clarifier Effluent	X		
Ponds #1 and #2 Effluent		X	
Ponds #3 - #6 Effluent			X
Cook's Slough, Upstream of Site			X
Cook's Slough, Downstream of Site			X
Soils			X

Sediments

Pond sediments were collected with a coring device which was driven manually into the sediment layer and capped on the upper end. The sample was raised to the pond surface and transferred as a slurry to sterile sample bottles. Sediments were held in wet ice during transport to the laboratory.

WASTEWATER ANALYSES

Virus Concentration Procedures

Laboratory Method--

Volumes of up to 10 liters were handled readily in the laboratory. Wastewater was placed in a vessel of convenient size and 100 mg/l of expanded bentonite was added, along with sufficient $CaCl_2$ to bring the wastewater to approximately 0.01M $CaCl_2$. The pH was adjusted to 6.0 with HCl and mixed for 30 minutes. After mixing, the virus-solids-bentonite complex was sedimented by low speed centrifugation and 10 to 60 ml of tryptose phosphate broth (TPO_4) added to the pellet for elution. Elution was accomplished by vortexing the TPO_4 solids-virus suspension for 5 minutes. The suspension was separated by centrifugation (3000 xg) and the TPO_4 containing the eluted viruses was assayed (see below).

The range of viral recovery for more than 55 separate field samples using this methodology was from 21 to 100%, and the mean was 53%. These were separate daily samples over a consecutive 49-day period. In a recent experiment, the recovery variability for one series of samples of oxidation ditch and trickling filter effluents ranged from 91% to 97%, well within expected variability. In two other studies (not Kerrville) identical samples processed in our Austin laboratory (now closed) and in our San Antonio laboratory resulted in similar corrected data, despite a considerable difference in recovery efficiency as described below (see Table 10).

TABLE 10. Comparison of Bentonite Concentration Technique Recovery Efficiency

	Sample #1			Sample #2		
	Austin	San Antonio	Mean	Austin	San Antonio	Mean
Corrected Virus (3d), pfu/l	35	46	41	263	243	253
Corrected Virus (5d), pfu/l	51	51	51	345	307	326
Concentration Efficiency, %	74	31	--	100	31	---

These results emphasize the importance of an efficiency of recovery control with seeded virus being a part of every assay, every day. The control seed of poliovirus is mixed into the water sample to be tested at approximately 10^2 pfu/ml, a dilution from stocks of at least 1:1000. At these viral input levels aggregation is not considered a problem. (Or, rather, verification of monodispersed titers is not justified.) It is likely that if a particular ionic milieu tends towards viral clumping, it will do so for both naturally-occurring and seeded virions.

After a 15-minute mixing period which allows dispersion of the poliovirus seed, the control sample is concentrated as described above. The total virus recovered from the eluate compared to the known amount of virus in the original sample allows calculation of the recovery efficiency.

Differences in reported recovery efficiencies between laboratories may be attributed to any of several variables including handling of the bentonite (expanded vs. nonexpanded clay), age and viral sensitivity of monolayer cultures employed for assay, and technician variability.

Field Batch System--

Prototype 1

In order to adapt the bentonite concentration procedure to a flow-through system, diatomaceous earth (Celite® 535 or 512) was added to retain the clay used as a viral adsorbent. The batch filtration system was prepared by placing a 15 cm diameter Buchner funnel on a five-gallon carboy. A #4 Whatman filter was placed on the funnel. Diatomaceous earth was employed at the precoat rate of 0.25 lb/ft^2 and body feed at rates up to 450 mg/l. Adequacy of filtration was determined by the observation of breakthrough (turbidity) of bentonite in the filtrate.

After filtration of the test water, the filter was lifted from the funnel and the filter mat scraped into a 250 ml plastic bottle. A sufficient volume of tryptose phosphate broth was added to the bottle to completely saturate the diatomaceous earth-bentonite-solids mass. The entire mixture was shaken vigorously for 5 minutes and packed in wet ice for shipment to the laboratory for assay. Separation of solids prior to assay was by low-speed centrifugation as described above.

Prototype 2

Subsequent efforts to increase the recovery of indigenous viruses from surface waters led to the modification of the first field batch system. Not only were flow rates limited by the presence of bentonite clay, but viral recoveries also were decreased due to increased amounts of diatomaceous earth used to maintain flux in the system.

In order to remove bentonite from the batch procedure, an alternate viral adsorbent was required. Developmental work on another study (EPA

Grant R-804474-02-0) had indicated the validity of viral concentration by retention of viruses on diatomaceous earth in the presence of divalent cation. Using the physical system described above, Celite 512 was applied as a precoat to a #4 Whatman filter at a rate of 0.03 gm/cm^2. Routinely, 20 liters of surface water (specifically pond water with high algal content) were concentrated through this precoat with the addition of body feed at a rate of 600 mg/l. Viruses were recovered from the diatomaceous earth-solids complex by elution in tryptose phosphate broth as described.

Standard Methods

Virus Concentrator--

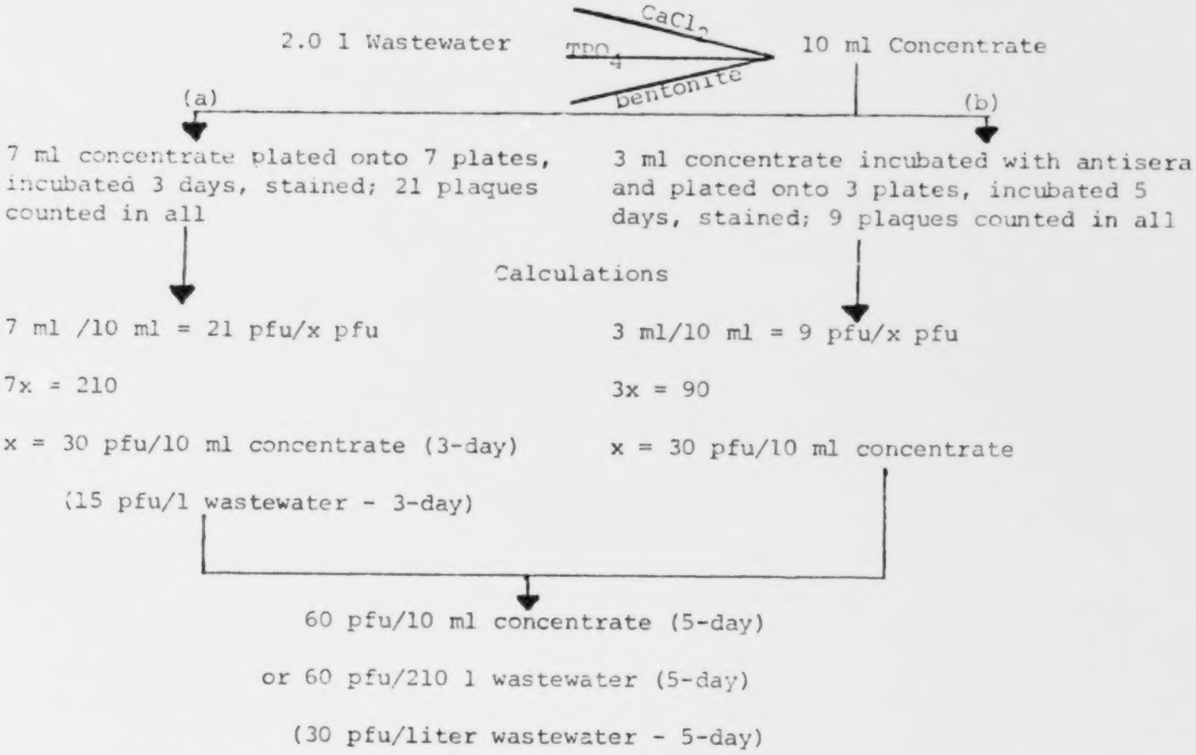
A portable virus concentrator developed by personnel of the USEPA Environmental Research Center, Cincinnati, was constructed. The viral adsorbent system utilized the Balston Grade C, 8 µm porosity filter cartridges. Viral concentration procedures outlined in *Standard Methods for the Examination of Water and Wastewater*, 14th edition (1976), were employed.

Using this field concentrator, minimally 100 gallon samples were processed routinely from wells having adequate flow. The relatively high particulate content of certain surface waters, especially ponds, limited volumes processed from these sources to 20 to 100 gallons.

Virus Assay

Initially, virus-containing concentrates had been assayed on HeLa cell monolayers for plaque-forming ability. Because poliovirus plaques increased in size rapidly in this system, it was difficult to determine the presence of other enteric viruses. Therefore, samples were divided into two portions, with two-thirds of the concentrate assayed for plaque enumeration after 3 days' incubation. The remaining one-third of the concentrate was mixed with pooled commercial poliovirus antisera (types 1, 2, 3) so as to make the concentrate 2% antisera. (This level of antiserum was sufficient to result in the neutralization of 95-99% of laboratory seed poliovirus.) After incubation at 37C for 30 minutes, the entire sample was plated onto HeLa monolayers, overlayered and incubated for 5 days before staining.

Final reported titers were calculated as given in the following example:


2.0 l Wastewater
CaCl2
TPO4
bentonite
10 ml Concentrate
(a)
(b)
7 ml concentrate plated onto 7 plates, incubated 3 days, stained; 21 plaques counted in all
3 ml concentrate incubated with antisera and plated onto 3 plates, incubated 5 days, stained; 9 plaques counted in all
Calculations
7 ml /10 ml = 21 pfu/x pfu
7x = 210
x = 30 pfu/10 ml concentrate (3-day)
(15 pfu/l wastewater - 3-day)
3 ml/10 ml = 9 pfu/x pfu
3x = 90
x = 30 pfu/10 ml concentrate
60 pfu/10 ml concentrate (5-day)
or 60 pfu/210 l wastewater (5-day)
(30 pfu/liter wastewater - 5-day)

Beginning in January, 1978, viral assays were performed by the plaque assay method utilizing HeLa and/or BGM monolayers on 100 mm plates. Samples were evenly divided between the two cell lines when both HeLa and BGM were used for viral detection. The growth medium was aspirated from the plates and the inoculum added, generally 1.0 ml per plate. Infected plates were rocked continuously on a Bellco Glass, Inc. rocker platform at room temperature for one hour. The plates were then washed with Hanks' Balanced Salt Solution containing penicillin G (500 units/ml) and streptomycin (250 µg/ml) for 20 minutes. The agar-based overlay media consisted of Eagle's Minimal Essential Media without phenol red containing 8% calf serum, 100 units of penicillin G per ml, 50 µg of streptomycin per ml, 25 µg of Gentamicin® per ml, and 0.5 µg of Fungizone® per ml. Three days post-inoculation a second agar overlay containing 30 µg/ml neutral red was applied to plates. The plates then were read each succeeding day and scored for plaques through 5 days. Care was taken not to expose the plates to light for extended periods of time.

BSC-1 tube cultures also were used during portions of the study in an attempt to recover viruses from well concentrates. These cells were grown in 16x25 mm glass tubes. Each tube was inoculated with 0.1 ml of suspect viral concentrate and subsequently monitored for a period of 21 days. The maintenance medium was changed approximately every fourth day.

All plates and tubes were incubated at 37C in a humidified atmosphere of 5% CO_2 in-air.

Viral Confirmation, Identification and Characterization

Possible viral isolates were picked from areas exhibiting cytopathic effect based on microscopic examination of the stained monolayer. The removal of plaque-like areas was accomplished by first removing the second overlay above the area of CPE. The entire plaque then was aseptically collected using a spatula. The sample was placed in 0.5 ml of Medium 199 containing 200 units of penicillin G per ml and 100 µg of streptomycin per ml and held at -76C to -80C until confirmation.

Confirmation of potential viral isolates was performed in homologous tube culture systems. Culture tubes were grown out to 50-75% confluence and inoculated with 0.1 ml of sample. After 48 hours, tubes were observed daily for evidence of CPE. At such time as CPE was observed, the sample was removed and frozen at -76C until the second tube culture passage. After seven days, all samples not showing CPE were harvested and passaged blind. Those isolates that demonstrated CPE after a second passage were reported as positive viruses.

Isolates were identified using the Lim-Benyesh-Melnick enterovirus typing pools. In those cases where viral breakthrough was observed at five days, identifications were confirmed using neutralization testing with monospecific antisera.

Because of the large number of viral isolates identified as poliovirus 1, further characterization of selected isolates were done using the reproductive capacity temperature (rct) marker. The limited thermal exposure test presented by Carp and Koprowski (1962) was followed. Poliovirus 1 field isolates were dilution plated on HeLa monolayers as previously described. One half of the plates at each dilution were incubated at 37C ± 0.5C in a humidified atmosphere of 5% CO_2. The remaining plates were placed in a 40.1C ± 0.2C humidified CO_2 incubator for 18 hours before transfer to the 37C incubator. Laboratory stocks of poliovirus 1 (Chat) and poliovirus 1 (Mahoney) were run as standard controls.

An r value for both laboratory attenuated and wild type virus strains and field isolates was calculated where:

$$r = \frac{\text{total number of plaques observed at 40C}}{\text{total number of plaques observed at 37C}}$$

Bacteriophage Assay

Samples to be assayed for coliphage content were plated using *Escherichia coli* K 13 as the host of choice. This organism was found to be the most sensitive in terms of total numbers of indigenous bacterial viruses detected in a variety of wastewater samples. Other coliform strains evaluated were *E. coli* B, *E. coli* $F_{100/C600}$, and *E. coli* K 12.

Appropriate sample volumes (0.1 - 1.0 ml) and 0.5 ml of an 18-hour broth culture of *E. coli* K 13 were added to 3.5 ml of liquified 1%

tryptose phosphate agar at 45C ± 1.5C. This inoculated volume was poured over a pre-formed tryptose phosphate 1.5% agar basal layer in a 100 mm petri dish. When firm, the plates were inverted and incubated at 35C for 8-18 hours, depending upon sample source. Soil and sediment analyses for coliphage were counted after 8 hours to avoid overgrowth of indigenous bacteria. Plaques were observed on an illuminated colony counter under 5X magnification.

Total and Fecal Coliform Assay

For coliform densities where samples greater than 0.1 ml were required, membrane filtration procedures using Gelman GN-6 filters as described in Standard Methods for the Examination of Water and Wastewater (1976), were utilized. However, for samples with high solids content where dilutions were required, a spread plating technique in either m-Endo agar LES (total coliforms) or m-FC agar (fecal coliforms) was utilized.

Results presented in Table 11 show representative results from field samples collected at the Kerrville site. Spread plating appeared to be comparable to membrane filtration for the recovery of total coliforms. Only slightly improved fecal coliform recoveries were observed using spread plating, perhaps due to a detrimental effect of filtration through the membrane matrix on these organisms.

Fecal Streptococci Assay

During the last year of the field study, fecal streptococci were added as a routine microbiological indicator of fecal pollution at the Kerrville irrigation site. A basis for the calculation and use of a fecal coliform to fecal streptococci ratio was set forth by Geldreich and Kenner (1969) and Feachem (1975). Subsequently, several investigators have used this measurement (or ratio) in evaluating environmental fecal pollution (DeMichele *et al*, 1974; Sayler *et al*, 1975; Davenport *et al*, 1975). As the field site at Kerrville was used as pasture for cattle grazing, the use of FC:FS ratios was necessary to differentiate pollution attributable to domestic wastewater irrigation from that due to animal wastes.

KF Streptococcus agar, prescribed by Standard Methods for the enumeration of fecal streptococci by plating procedures, and m-Enterococcus agar, suggested by M. Neal Guentzel, The University of Texas at San Antonio, were compared using identical field samples. Appropriate dilutions of water samples from three different sources were spread plated in triplicate. After incubation at 35C for 48 hours, plate counts were taken, and selected random colonies were inoculated onto bile esculin slants. (Bile esculin is a differential biochemical that confirms streptococci as being of fecal origin.) Table 12 shows the results of these efforts.

M-Enterococcus agar yielded superior plate counts and a higher percentage of confirmed fecal streptococci from all water sources.

Therefore, this medium was chosen for routine enumeration of fecal streptococci. As described above, for organism densities requiring samples greater than 0.1 ml, membrane filtration procedures were employed. However, for samples with fecal streptococci densities requiring less than 0.1 ml, spread plating was used as the plating method.

TABLE 11. . Comparison of Membrane Filtration and Spread Plating Techniques

Sample	Total Coliform (cfu/100 ml) Membrane Filtration	Total Coliform (cfu/100 ml) Spread Plating	Fecal Coliform (cfu/100 ml) Membrane Filtration	Fecal Coliform (cfu/100 ml) Spread Plating
1	$1.7x10^{6}$	$1.4x10^{6}$	$2.0x10^{5}$	$2.5x10^{5}$
2	$1.9x10^{6}$	$2.0x10^{6}$	$2.1x10^{5}$	$2.7x10^{5}$
3	$8.0x10^{5}$	$8.9x10^{5}$	$7.0x10^{4}$	$9.5x10^{4}$
4	$5.7x10^{5}$	$5.1x10^{5}$		
5	$3.6x10^{5}$	$3.8x10^{5}$		

TABLE 12. Comparison of Fecal Streptococci Isolations on Selective Media

Media & Sample	Fecal Streptococci counts (cfu/100 ml)	# colonies tested on bile esculin	% confirmed fecal streptococci
KF Streptococcus Agar			
tail water pond	$3.0x10^{1}$	8	100
lysimeter water	$2.4x10^{4}$	42	95
well water	$8.0x10^{1}$	9	88
m-Enterococcus Agar			
tail water pond	$8.3x10^{1}$	20	100
lysimeter water	$1.8x10^{4}$	37	95
well water	$2.6x10^{2}$	28	100

Bacteriological Screen

During the course of this study, a bacterial screen for overt as well as opportunistic human pathogens was conducted on wastewater samples at both Kerrville and Uvalde. In addition to indicator organisms, screening procedures were run to optimize recovery of a variety of gram-negative enteric bacteria.

Total Plate Count--

Serial dilutions of the sample were prepared in sterile phosphate buffered saline. A soft-agar overlay (tryptose-phosphate with 0.3% Agar) and 1 ml of the appropriate dilution of sample were pour-plated over five plates of plate count agar. Incubation was at 35C for incubation period of 24-48 hours. Only plates showing 30-300 colonies were counted.

Mycobacteria--

Samples were treated for 20-35 minutes with 500 ppm benzalkonium chloride (Zephiran), diluted and plated to the surface of Middlebrook's 7H10 agar plates. Plates were incubated at 37C in a 5% CO_2 atmosphere and examined over a period of one month for the appearance of typical Mycobacteria colonies. Suspect colonies were identified by examination of stained (Ziehl-Neelson) smears for acid-fast bacilli.

Staphylococci--

Appropriate dilutions of the wastewater sample were spread plated onto Mannitol Salt Agar. Typical staphylococcal colonies showing a yellow zone of mannitol fermentation were isolated for confirmation of identity by observation of gram-positive cocci.

Fluorescent Pseudomonads--

Appropriate dilutions of sample were plated onto Pseudomonas Agar F (Difco) and Cetrimide Agar (Difco). Following incubation at 35C for 24 hours and subsequent exposure to fluorescent light for 24 hours, the number of fluorescent pseudomonads was determined by counting the number of fluorescing colonies observed under long-wave ultraviolet light in the dark.

Enterobacteriaceae--

Salmonella species: Enrichment for Salmonellae was done by inoculating Selenite and Tetrathionate broths with various sample volumes (10-25 ml). The broths were incubated at 35C for 24 hours. After enrichment, aliquots were streaked for isolated colonies onto XLD, Hektoen, and SS Agars. Identification of suspect colonies was accomplished by use of the Enterotube®, a biochemical and computer-coded identification system for *Enterobacteriaceae*.

Shigella species: Enrichment for Shigallae was done by inoculating GN broth (BBL) with various volumes of sample (10-25 ml). The GN Broth was incubated at 35C for 24 hours. After enrichment, aliquots were plated for isolated colonies onto XLD agar. Identification of suspect colonies was accomplished by use of the Enterotube®, a biochemical and computer-coded identification system for *Enterobacteriaceae*.

Yersinia enterocolitica: Isolation of *Yersinia* was attempted by selective enrichment by inoculation of sample into isotonic saline containing 25 mg/l of potassium tellurite. Enrichments were incubated at SC for 7 days. Aliquots from enrichment media were streaked onto SS agar and incubated at room temperature (25C) for 2 days. Colonies were identified using the Enterotube® system.

Other Enterobacteriaceae (i.e., *Escherichia, Citrobacter, Klebsiella, Enterobacter, Serratia, Proteus*, and *Providencia*): These organisms were enumerated by isolating and subculturing colonies from samples dilution plated to moderately selective (XLD & Hektoen) and highly selective (Bismuth Sulfite & SS) enteric plating media. Two to three representative of every colony type observed were subcultured and subsequently identified using the Enterotube® system.

Other Gram-Negative Enteric Bacteria (*Aeromonas, Flavobacterium, Pasteurella, Plesiomonas*, and *Pseudomonas* sp.): Colonies isolated from the non-selective enteric plating media (i.e., EMB & MacConkeys agars) were identified using the Oxi/Ferm® tube, a standardized system specificially designed for the identification of oxidative-fermentative gram-negative rods. This identification scheme was utilized only at the Kerrville site.

Chemical and Physical Analyses

All samples for wastewater analyses were stored at 4C and handled in accordance with guidelines shown in Table 13. Chemical analyses were performed within time degradation limits established by studies performed on selected samples from the Kerrville and Uvalde sites. Additionally, recovery tests utilizing various sample dilutions and additions of known standards to samples were conducted routinely to eliminate erroneous data due to interferences.

Analyses of field samples for five-day biological oxygen demand (BOD_5), pH, turbidity, specific conductance, total suspended solids (TSS) and volatile suspended solids (VSS) were conducted on unpreserved samples in accordance with the Environmental Protection Agency's Methods for Chemical Analyses of Water and Wastes (1974) and Standard Methods for the Examination of Water and Wastewater (1976). Samples for total organic carbon (TOC) analysis were acidified with either hydrochloric or sulfuric acid and subsequently measured using methodology specified by the EPA.

Samples for other chemical analyses were preserved by the addition of 40 mg/l of mercuric chloride. These tests included manual procedures for total and 'reactive' (soluble) phosphorus and automated procedures for determination of nitrite nitrogen, ammonia nitrogen, and total Kjeldahl nitrogen. Nutrient analyses were performed utilizing procedures

TABLE 13. Holding Procedures for Routine Chemical/Physical Analysis

Analysis	Remarks
pH	No hold time, field measurement
turbidity	Measure within 4 days, hold @ 4C
conductance	Relatively stable, read at room temperature
solids	Measure within 4 days, hold @ 4C
BOD_5	Hold @ 4C, set up within 24 hours
TOC	Hold @ 4C for 2 weeks with acidification
Nitrogen	Hold time of 2 weeks with preservation ($HgCl_2$) @ 4C
TKN	
NH_3	
NO_3/NO_2	
Phosphates	Hold time of 2 weeks with preservation ($HgCl_2$) @ 4C
Total P	
Soluble P	

reported by Fruh *et al* (1975) adapted from EPA and Technicon AutoAnalyzer® methodologies.

Additionally, samples of groundwater collected from each of the monitoring wells at the Kerrville site were analyzed for Pb, Cd, Fe, Cr, Cu, Mn, Ni, Na, K, Ca, Mg, and Zn. Atomic absorption analysis was performed using a Perkin-Elmer Model 460 dual-beam, background corrector-equipped atomic absorption spectrophotometer.

Groundwater samples collected in the field were fortified immediately with concentrated nitric acid to reduce analytic loss due to adsorption on the collection container walls. Laboratory preparation included additional acidification and dilution or concentration depending on concentrations of metals present. Samples analyzed for Na and K were treated additionally to control ionization interferences.

SOIL/SEDIMENT ANALYSES

Bacteriophage Assay

Approximately 50 gm of each soil sample were placed in a 250 ml centrifuge bottle with 100 ml tryptose phosphate broth and mixed in a controlled environment incubator-shaker at 300 rpm for 30 minutes. The samples then were centrifuged at 1500 xg for 5 minutes. A supernatant volume was removed for bacteriophage assay as described above. The pellet was collected in deionized water and dried at 103C. The dried soil weight was used for reporting the number of viruses recovered per gram of soil. Alternately, soil moisture values were used to calculate phage recoveries.

Enteric Virus Recovery

Initial attempts to recover animal viruses from soil used the protocol described above for bacteriophage work. The procedure was modified in that samples were centrifuged at 10,000 xg for 30 minutes to pack soil solids. During subsequent field studies, it became obvious that larger amounts of soil should be sampled in an effort to detect enteric viruses. Therefore, an alternate elution and reconcentration procedure was developed. Viral elution from Kerrville soils was optimized to allow reconcentration of soil eluates. Poliovirus 1 (Chat) suspended in secondary effluent was introduced into replicate 50 gm soil samples contained in 7 cm Buchner funnel "bins". After the bins had drained, each filtrate volume was assayed for viral infectivity to allow calculation of the viral retention by the soil samples. Each soil sample was then subjected to a combination of elution-reconcentration schemes.

Two general classes of eluting media were compared: high pH glycine buffer and high soluble organic medium as typified by beef extract and tryptose phosphate broth. Reconcentration procedures were dictated by the type of elution medium employed. Glycine buffer eluates were concentrated by adsorption on Cox filters followed by reelution of viruses as outlined in the reconcentration procedure for microporous filter concentration of enteric viruses (Standard Methods, 14th edition). Organic flocculation was done as described by Katzenelson, et al (1976). Hydroextraction of samples was accomplished according to the method of Wellings, et al (1975).

Results from this experiment (Table 14) indicated that elution with 3% beef extract, pH 9, followed by organic flocculation resulted in the best overall recovery of poliovirus from this soil. Therefore, this technique was utilized in attempts to recover viruses from 500 gm soil samples.

During soil sampling, ten 50 gm samples were processed. Each sample was homogenized in a Waring blender with 150 ml of 3% beef extract for 3 minutes. Samples then were centrifuged at 1500 xg for 10 minutes

TABLE 14. Poliovirus Recovery from Kerrville Soil.

Elution[α]-Reconcentration System	Virus Eluted (%)[β]	Virus Reconcentrated (%)[γ]	Total Recovery (%)[β]
1. Distilled H_2O Bentonite	23	100	23
2. 0.05 M Glycine, pH 11.5 - Cox filter adsorption	65	58	38
3. 0.25 M Glycine, pH 11.5 - Cox filter adsorption	61	63	38
4. 3% beef extract, pH 9 - organic flocculation	73	70	51
5. Tryptose phosphate broth - PEG hydroextraction	56	48	27

[α] Soil + eluate homogenized in a blender for 1 minute, followed by low speed centrifugation to remove solids.

[β] % of pfu retained soil bin.

[γ] % of pfu recovered in soil eluate.

and supernatants pooled. The eluate volume was concentrated by organic flocculation. Final volumes for assay ranged from 50 - 80 mls.

Sediment samples were treated as soil samples. Sediments, taken as a slurry, were homogenized with equal volumes of 3% beef extract for three minutes. Samples were centrifuged by 1500 xg for 10 minutes. The supernatant was reconcentrated by organic flocculation as described by Katzenelson (1976). Final volumes ranged from 30-50 mls.

Total and Fecal Coliform Assay

The methodology initially used to assay for coliform levels in soils depended upon elution of these organisms from the soil sample. In addition to highly variable elution, low-speed centrifugation (necessary to remove particulates prior to membrane filtration) introduced yet another variable. Total bacterial counts based on direct plating of soil slurries and post-centrifugation counts based on plating of soil eluates attest to this variability (see Table 15). For this reason, standard multiple tube fermentation was used as a more reproducible method to enumerate coliform organisms in soil and sediment samples.

An aqueous suspension of either soil or sediment was mixed in a laboratory shaker at 300 rpm for 30 minutes. Appropriate dilutions then were inoculated into the presumptive MPN test medium (Lauryl Tryptose Broth) using the procedures described in Standard Methods (1976). Subsequently, the samples were transferred into confirmation test media, Brilliant Green Bile, 2% and EC Medium, for total and fecal coliform MPN determinations, respectively.

Reported values were calculated from MPN tables and dry weight determinations of soil or sediment taken at 103C.

TABLE 15. Enumeration of Total Aerobic Bacteria from Soils

Soil		Bacterial Colonies/gm of Soil		
Area	Sample	Direct Plate	Eluted	% Eluted
1	A	8.4×10^6	1.8×10^6	21
1	B	2.4×10^6	1.9×10^5	8
2	A	2.2×10^7	8.6×10^6	39
2	A	1.5×10^7	2.2×10^6	15
3	A	2.0×10^7	1.9×10^5	1
3	B	1.0×10^7	1.8×10^5	2

Fecal Streptococci Assay

The multiple-tube technique for the enumeration of fecal streptococci as described in Standard Methods (1976) was used during this study. Verification of the confirmed test procedure was done by transferring a loopful of growth from positive EVA tubes to Bile Esculin slants. Results of this testing from three separate soil samples are presented in Table 16. Based on these observations, the MPN procedure as used probably results in somewhat lower numbers. However, for the routine detection of fecal streptococci in samples with a high solids content and low indicator organism density, the multiple-tube technique was the most consistent procedure available.

Samples were handled as aqueous suspensions (as described for coliform indicator organisms).

TABLE 16. Enumeration of Fecal Streptococci in Soil Samples

Soil Sample	Fecal Streptococci (MPN/100 ml) EVA broth	Bile Esculin
1	1.7×10^5	2.2×10^5
2	1.6×10^6	$\geq 2.4 \times 10^6$
3	3.5×10^3	7.0×10^4

Chemical/Physical Analyses

Sieve Analysis--

Fractionation of soils was by standard, mechanical sieve analysis employing sieves sized from 0.05 to 1.4 mm. From these data, soil aggregate distribution curves were prepared.

Moisture Content--

Moisture content was determined in the laboratory by oven drying to 103C. Soil moisture cells (Soil Test, Inc.) manufactured as two metal plates separated by a fiberglass binding were installed adjacent to each of the lysimeters. The design of these soil moisture cells provided a coupling so that resistance increased as ohms varied with soil moisture content. The soil moisture cells must be calibrated for each soil on the basis of a curve relating soil moisture to resistance.

Chemical Analysis--

Preparatory to analyses for cation exchange capacity (CEC) and organic carbon content, soils were dried overnight at 103C and passed through a 16 mesh (1 mm) sieve.

CEC determinations were performed using the method outlined by Jackson, 1958.

The percentage of organic carbon in soils was determined according to Hesse (1971) except that the procedure was modified by using a phenanthroline indicator titrating to a turquoise blue end point.

Measurements of pH were taken on wet samples diluted 50% wt/wt with distilled water and equilibrated for 30-60 minutes.

Physical Properties--

Soil particles are discrete units of diverse composition, size, and shape. Soil particles may be classified by determining their size (generally the width of the smallest square opening through which the particles can pass) and applying the relative proportions of the particle sizes present to a textural classification such as sand, silt and clay. Soil particle sizes are classified by the U.S.D.A. as >2 mm=gravel, 0.5-2 mm=sand, 0.001-0.05 mm=silt, <0.002 mm=clay. Soil samples from the field were analyzed by standard dry sieving methods. Organic material was removed mechanically and by oxidation with hydrogen peroxide. Clay fractionation was performed by pipette analysis, utilizing a modification of Stokes principle.

Atomic Adsorption Analyses--

Soil samples were collected from each major soil series persent at the Kerrville site. The soil samples were analyzed using the Perkin-Elmer Model 460 atomic absorption spectrophotometer for Pb, Co, Fe, Cr, Cu, Mn, Ni, Na, K, Cd, Mg and Zn.

Soil samples were collected from the upper 0.2M of the soil profile at each sampling site. Each sample was thoroughly mixed and stored in air-tight containers for transport to the laboratory. In the laboratory the soil samples were sieved to remove large rocks and the organic debris was removed by mechanical extraction. The samples were ground and crushed using a morter and pestle and then oven-dried at 60C. Samples were digested with 5N nitric acid, centrifuged and the leachate analyzed.

X-Ray Diffraction, Qualitative Analyses--

Each major soil series at the Kerrville site was analyzed for mineralogical composition of the silt and clay size fraction. As the silt and clay size fraction present in a soil plays a major role in determining the physical and chemical characteristics of that soil, a knowledge of the mineralogical composition is necessary to assist in evaluating the soil for wastewater disposal purposes.

The term "clay" has no generic significance. Clays are materials which are produced by the weathering of other geologic materials and by deposition as sediment. Silt and clay are primarily particle size terms. The size range used in this report is silt (0.05-0.002 mm) and clay (finer than 0.002 mm).

Soil samples were dispersed by sonication in distilled water. Each sample was wet-sieved through a 325 mesh sieve. The portion of the sample passing through the sieve then was sonicated and centrifuged at 10,000 rpm (7800x g) for 5 minutes. Samples then were redispersed by sonication in distilled water and centrifuged for 90 sec at 1000 rpm (approximately 80x g). At this point, the separation of silt from clay was made, with the clays being decanted. The sediment remaining was applied to glass slides and air-dried. The decanted volume was sonicated and centrifuged at 10,000 rpm for 5 minutes. The clay sediment was applied to glass slides and air-dried.

A Siemens X-ray diffractometer was used for all samples. The scanning speed was 2.5° 20 per minute and chart speed was 2 inches per minute. All samples were run from 2° to 64° 20. After obtaining a diffraction pattern for each sample each peak was indexed and converted to the appropriate d sparings. ASTM powder data file cards then were referenced to identify the minerals present.

SPECIAL IRRIGATION STUDY

Studies of the effect of intensive irrigation were undertaken at the Kerrville field site. Arrangements were made with local personnel to manage the area around selected lysimeters for ten-day study periods. All irrigation was withheld from the area for 72 hours prior to the collection of background soil samples. At this time lysimeters also were evacuated. Normal cycles of irrigation performed on a 12-hour spray period followed by a 36-hour drying period then were initiated.

Samples for laboratory analyses were obtained on a daily basis. Lysimeter samples were collected after approximately 6 hours of irrigation. Grab samples from the effluent line at the irrigation pond also were taken at this time. Soil samples were collected after approximately 18 hours of drying.

SECTION 8

RESULTS AND DISCUSSION

Early second year study efforts focused on developing a data base at both the Kerrville and Uvalde sites. The research plan was designed to accumulate significant data on treatment effectiveness prior to and during lysimeter and monitoring well installation, thus permitting a shift of emphasis to studies of the lysimeters and wells during the final study year. Further, it was thought important to develop the same types of data for the soils prior to initiation of a regular irrigation schedule as would be obtained in the final year.

WASTEWATER ANALYSES

Chemical and Physical Analyses

Kerrville--

Table 17 presents the mean results of wastewater chemical and physical analyses at all sampling points at the Kerrville site. The wastewater is of moderate strength (BOD_5 = 175 mg/l) and the treatment quite effective as shown by 92% removal of BOD_5 by both the oxidation ditch system and the trickling filter system. These results were not unexpected if one considers that the treatment plant currently is operating at approximately 70% of its design capacity of 2.25 mgd. The average solids carry-over from the oxidation ditch system was comparatively high but this was due to sludge handling difficulties at the plant during the Winter of 1976-77.

Total organic carbon, BOD_5 and solids concentrations found in the irrigation pond were in keeping with the effectiveness of treatment. Considerable variation in these parameters, however, was observed in the tailwater pond. The tailwater pond variations were attributed to the periodic presence of extensive duck and algal populations.

Statistically significant changes in Third Creek's chemical properties, such as increases in orthophosphate and nitrate concentrations, were seen downstream of the irrigation site. These changes may be the result of minor seepage of wastewater through the dike protecting the creek and/or from groundwater discharges to the creek. However, the observed increases do not represent a marked degradation of water quality.

Uvalde--

Table 18 presents the mean results of wastewater chemical and physical analyses for all sampling points at the Uvalde site. Here the

TABLE 17. Mean Results of Wastewater Chemical/Physical Analyses, Kerrville Site

Sample Point	Total Organic Carbon (mg/l)	BOD_5 (mg/l)	Suspended Solids (mg/l)		Ortho-Phosphate (mg/l-P)	Nitrogen Forms (mg/l-N)				pH (Units)
			Total	Volatile		NH_3	NO_3	NO_2	TKN	
Raw Wastewater*	107	175	170	129	7.6	21.0	0.16	0.09	26.7	7.50
Oxidation Ditch Effluent*	19	14	20	15	6.6	9.30	0.84	<0.03++	11.0	7.78
Trickling Filter Effluent*	23	14	18	13	8.1	1.77	19.6	0.24	3.60	7.98
Irrigation Pond†	21	26	39	30	5.4	2.91	4.88	1.95	6.21	8.16
Tailwater Pond†	25	20	48	30	2.1	0.23	0.48	<0.31σ	1.90	9.02
Third Creek, Upstream†	9.4	<1.6**	7.4	4.6	<0.06‡	<0.03β	1.29	<0.02α	<0.33αα	7.92
Third Creek, Downstream†	8.0	<1.3π	7.6	5.1	0.4	<0.04ε	2.66	<0.03μ	<0.47μμ	7.84

* Values derived from sixteen samples for most analyses.

† Values derived from a minimum of twenty samples.

‡ Values ranged from <0.01-0.09 mg/l.

σ Values ranged from <0.01-2.20 mg/l.

α Values ranged from <0.01-0.10 mg/l.

μ Values ranged from <0.01-0.10 mg/l.

β Values ranged from <0.01-0.10 mg/l.

ε Values ranged from <0.01-0.09 mg/l.

** Values ranged from <1.0-3.0 mg/l.

π Values ranged from <1.0-3.0 mg/l.

++ Values ranged from <0.01-0.07 mg/l.

αα Values ranged from <0.20-0.80 mg/l.

μμ Values ranged from <0.20-1.40 mg/l.

TABLE 18. Mean Results of Wastewater Chemical/Physical Analyses, Uvalde Site*

Sample Point	Total Organic Carbon (mg/l)	BOD_5 (mg/l)	Suspended Solids (mg/l)		Ortho-Phosphate (mg/l-P)	Nitrogen Forms (mg/l-N)				pH (Units)
			Total	Volatile		NH_3	NO_3	NO_2	TKN	
Raw Wastewater	49	79	73	59	3.1	11.30	0.82	0.31	8.54	7.67
Trickling Filter Effluent	23	35	36	29	3.3	9.26	1.44	0.50	8.64	8.06
Pond #1	18	30	38	27	4.4	9.70	0.81	0.29	9.56	8.16
Pond #2	20	39	40	34	4.4	9.00	0.55	0.10	10.44	8.14
Pond #3	22	28	25	20	4.5	9.65	0.65	0.08	9.13	8.00
Pond #4	22	23	32	30	4.4	10.48	0.43	0.07	10.31	8.02
Pond #5	21	18	22	20	3.8	8.35	0.49	0.09	9.44	7.94
Pond #6	18	18	26	20	3.2	7.30	0.53	0.25	7.39	8.29
Cook's Slough, Upstream	16	7	48	18	1.2	1.03	0.37	0.17	0.91	7.83
Cook's Slough, Downstream	21	6	44	21	0.8	1.25	2.30	0.23	1.76	7.69

* Values derived from six to eight samples.

wastewater was relatively weak as reflected by the BOD_5 (79 mg/l), total suspended solids (73 mg/l), the orthophosphate (3.1 mg/l) and nitrogen forms. Local observations indicate that considerable infiltration exists in the Uvalde sewage collection system. This would provide some basis for the observed low strength of the wastewater. The fact that only 56% of the BOD_5 and 51% of the total suspended solids were removed by the time the wastewater passed the trickling filter was most likely a direct result of the extremely high hydraulic loading on the unit processes in the treatment train. It should be noted, however, that the six ponds are a part of the total treatment system and that by the time the wastewater passes through the sixth pond, 77% of the BOD_5 has been removed.

The collected data do not reflect any major negative impact of the wastewater treatment plant discharge on Cook's Slough. In fact, except in the case of the nitrogen forms, the water quality as measured by these parameters exhibited no significant changes as a result of the treatment facility.

Bacteriological Assay

Kerrville--

Table 19 contains the mean results of total and fecal coliform determinations for all sampling points at the Kerrville site. These data were consistent with the chemical data in terms of treatment effectiveness throughout the system.

There was one interesting observation, however. Although the total coliform and fecal streptococci concentrations downstream of the treatment facility were higher than they were upstream on Third Creek, the fecal coliform concentrations were not. In fact, there was probably no significant difference between the fecal coliform concentrations upstream and downstream of the treatment facility on Third Creek.

When examining the fecal coliform-fecal streptococci ratios (FC:FS) shown in Table 19, one should not conclude that the majority of human fecal material in Third Creek was present prior to the stream entering the site, although Feachem (1975) has suggested that due to differential die-off, such a falling FC:FS ratio indicates fecal material of human origin. In this situation, however, the falling ratios can be attributed to a significant increase in the absolute numbers of fecal streptococci along the stretch of Third Creek studied. The exact source of this increase is not known, but may be associated with either wastewater from the irrigation pond or the treatment plant effluent which is piped beneath the creek under a significant hydraulic head. If the latter were the source, however, one would expect higher concentrations of both total coliform and fecal coliform at the downstream sampling point.

Uvalde--

Table 20 includes the mean results of total and fecal coliform determinations for all sampling points at the Uvalde site. These data are reflective of the chemical data, also.

It is interesting to note that the total and fecal coliform concen-

TABLE 19. Mean Results of Wastewater Bacteria and Bacteriophage Analyses, Kerrville Site*

Sample Point	Total Coliform (cfu/100 ml)	Fecal Coliform (cfu/100 ml)	Fecal Streptococci (cfu/100 ml)	Ratio FC:FS	Bacteriophage (pfu/l)
Raw Wastewater *	$1.7x10^8$	$4.4x10^7$	---	---	$8.6x10^6$
Oxidation Ditch Effluent*	$9.6x10^6$	$2.4x10^6$	---	---	$7.7x10^4$
Trickling Filter Effluent*	$1.3x10^7$	$2.5x10^6$	---	---	$1.3x10^6$
Irrigation Pond+	$4.9x10^5$	$1.2x10^5$	$2.7x10^3$	44	$2.0x10^4$
Tailwater Pond+	$7.0x10^3$	$5.5x10^2$	$6.8x10^2$	0.81	$< 1.0x10^0-1.5x10^4$α
Third Creek, Upstream+	$3.0x10^3$	$4.9x10^2$	$1.6x10^2$	3.09	$< 5.0x10^{-1}-1.2x10^4$α
Third Creek, Downstream+	$7.0x10^3$	$3.0x10^2$	$1.1x10^3$	0.27	$< 8.8x10^{-1}-2.4x10^4$α

* Values derived from sixteen samples.

+ Values derived from a minimum of twenty samples.

α Range

TABLE 20. Mean Results of Wastewater Bacteria and Bacteriophage Analyses, Uvalde Site*

SAMPLE POINT	TOTAL COLIFORM (cfu/100 ml)	FECAL COLIFORM (cfu/100 ml)	BACTERIOPHAGE (pfu/l)
Raw Wastewater	7.0×10^7	1.1×10^7	4.5×10^6
Trickling Filter Effluent	1.8×10^7	3.5×10^6	2.5×10^6
Pond #1	1.2×10^7	2.7×10^5	4.3×10^5
Pond #2	1.3×10^6	3.4×10^5	2.6×10^5
Pond #3	4.8×10^5	1.1×10^5	2.5×10^5
Pond #4	4.0×10^5	9.2×10^4	1.2×10^5
Pond #5	4.4×10^5	6.8×10^4	4.2×10^4
Pond #6	2.5×10^5	5.2×10^4	8.8×10^3
Cook's Slough, Upstream	2.8×10^3	$< 3.3 \times 10^{-1} - 3.2 \times 10^3$ +	$< 2.4 \times 10^0 - 4.5 \times 10^3$ +
Cook's Slough, Downstream	5.3×10^3	$< 3.3 \times 10^{-1} - 2.5 \times 10^3$ +	$< 2.4 \times 10^0 - 1.6 \times 10^4$ +

* Values derived from eight samples

+ Range

trations of the Uvalde raw wastewater were lower than those observed at Kerrville. Again, this reflects the lower strength of the Uvalde wastewater.

Bacteriological Screen

Table 21 presents the data from a single bacteriological screen of the raw wastewater and irrigation pond sampling sites at Kerrville as well as the raw wastewater at Uvalde. As the bacteriological screen used at each site differed in scope, the results are not directly comparable. However, the organisms detected by these screens attest to the presence of a wide variety of potential-pathogenic and/or opportunistic bacterial species at each site.

Virus Assay

Kerrville--

Table 19 includes a summary of results of analyses for coliphage for most sampling points at the Kerrville site.

Net bacteriophage reductions throughout the treatment system were not as dramatic as were the total and fecal coliform reductions. In part, this was because the oxidation ditch was much more efficient in coliphage removal than was the trickling filter. The minor increase in bacteriophage concentrations in Third Creek may be the result of small amounts of wastewater penetration through the dike. This observation is consistent with the data obtained for selected chemical parameters.

Enterovirus concentrations observed in the raw wastewater have been extremely variable throughout the study period (Tables 22 and 23). The initial low levels observed were thought to be related to some component in the wastewater which was either virucidal or interfered with the recovery and/or assay of indigenous virions by bentonite concentration. Experimentation with stock virus did not support this theory. A more plausible explanation involves the demography of Kerrville. The community has a large contingent of retired individuals, who are not likely to be shedding poliovirus, usually the most common enterovirus isolated.

Reduction of enteroviruses through the oxidation ditch system ranged from 61% to 99.9%. Reduction through the trickling filter system ranged from 59% to 95%, which appears to be somewhat high but is consistent with mean bacteriophage reductions (see Table 19).

Virus levels observed in the effluent from the oxidation ditch system were always low (see Table 23). Further, virus levels observed in the trickling filter system effluent paralleled the variability observed in raw wastewater levels. Removal efficiencies were high, generally, in both systems. It is probable that this was due to both hydraulic and organic underloading of the facilities. As can be seen in Table 23, there does not appear to be any correlation between virus levels observed and flow or virus concentration efficiency.

TABLE 21. Bacteriological Screen, Kerrville and Uvalde Sites

Organism	Colony-Forming Units/100 ml Kerrville Site Raw Wastewater (24-hr Composite)	 Kerrville Site Irrigation Pond (Grab Sample)	 Uvalde Site Raw Wastewater (24-hr Composite)
Total coliform	6.8×10^{7}	1.4×10^{5}	1.4×10^{8}
Fecal coliform	2.3×10^{7}	8.1×10^{4}	4.2×10^{6}
Fecal streptococci	9.2×10^{5}	2.1×10^{3}	1.4×10^{5}
Fluorescent Pseudomonads	7.8×10^{6}	3.0×10^{3}	1.7×10^{6}
Mycobacteria	1.2×10^{5}	1.0×10^{2}	CS
Staphylococci	1.2×10^{5}	8.7×10^{3}	9.3×10^{4}
ENTEROBACTERIACEAE			
Citrobacter diversus	1.3×10^{6}	ND[†]	ND
Citrobacter freundii	8.0×10^{6}	6.7×10^{2}	1.0×10^{5}
Enterobacter aerogenes	ND	$\geq 3.3\times10^{0}$ (E)	3.3×10^{5}
Enterobacter agglomerans	2.7×10^{6}	2.0×10^{3}	1.0×10^{6}
Enterobacter cloacae	1.5×10^{7}	1.1×10^{4}	1.4×10^{6}
Enterobacter hafniae	ND	$\geq 3.3\times10^{0}$ (E)	ND
Escherichia coli	6.0×10^{6}	5.3×10^{3}	2.0×10^{6}
Klebsiella ozaenae	8.3×10^{6}	6.7×10^{2}	ND
Klebsiella pneumoniae	1.5×10^{7}	1.3×10^{4}	6.6×10^{5}
Proteus mirabalis	$\geq 6.7\times10^{0}$ (E)	6.7×10^{0}	ND
Proteus morganii	ND	3.3×10^{0}	ND
Proteus rettgeri	8.3×10^{6}	ND	ND
Providencia alcalifaciens	$\geq 3.3\times10^{0}$ (E)	ND	ND
Providencia stuartii	$\geq 3.3\times10^{0}$ (E)	ND	ND
Salmonella sp.	ND	$\geq 6.7\times10^{0}$ (E)	3.3×10^{4}
Serratia liquefaciens	1.3×10^{6}	6.7×10^{2}	ND
Serratia marcescens	ND	$\geq 3.3\times10^{0}$ (E)	ND
OTHER GRAM-NEGATIVE BACTERIA (Kerrville Site Only)			
Aeromonas hydrophilia	1.7×10^{8}	1.3×10^{4}	
Flavobacteria	6.7×10^{5}	ND	
Pasteurella sp.	8.3×10^{6}	ND	
Plesiomonas shigelloides	3.3×10^{6}	4.0×10^{3}	
Pseudomonas aeruginosa	6.7×10^{5}	ND	
Pseudomonas fluorescens	1.3×10^{6}	1.3×10^{3}	
Pseudomonas putida	1.3×10^{6}	ND	
Pseudomonas stutzeri	1.7×10^{7}	ND	

CS = Contaminated Sample
† ND = None Detected
(E) Isolated from Enrichment Sample

TABLE 22. Results of Wastewater Enterovirus Assay, Kerrville Site*

Sample Point	Range of Enterovirus Concentrations Recovered (pfu/l)		Remarks
	3-Day	5-Day	
Raw Wastewater	5.6-780	5.6-1100	
Oxidation Ditch Effluent	<0.3-5.7	<0.2-8.8	
Trickling Filter Effluent	0.7-320	0.6-350	
Irrigation Pond	<0.15-6.0	<0.003-22	Several positive samples; most samples below detection sensitivity.
Tailwater Pond	---	---	All values below detection sensitivity which ranged from 0.01 to 2.2 pfu/l.
Third Creek, Upstream	<0.01-0.66	<0.003-0.66	Detection sensitivity ranged from 0.003 to 2.2 pfu/l; single positive sample identified as 3 poliovirus 1 isolates.
Third Creek, Downstream	<0.1-0.23	<0.003-0.23	Detection sensitivity ranged from 0.003 to 2.2 pfu/l; one sample showed positive CPE, however, it was not confirmed.

* Values derived from a minimum of twenty samples; in one case, a grab sample was collected from all sample points.

TABLE 23. Effectiveness of Wastewater Treatment Processes in Reducing Enterovirus Recovery (Selected Sampling Days), Kerrville Site

Sampling Point	Enterovirus Recovered (pfu/l, 3 day) and Treatment Effectiveness (%)									
	Sampling Date									
	9/15-16/76	11/9-10/76	12/9-10/76	2/8-9/77	3/7-8/77	4/5-6/77	5/4-5/77	7/11-12/77	8/11-12/77	11/16-17/77
Raw Wastewater	9.1	6.8	31	8.0	7.0	5.6	70	780	910	34
Oxidation Ditch Effluent	0.7	<0.3	2.0	1.3	0.5	2.2	1.8	1.0	5.7	1.2
(% Reduction)	(92%)	(>96%)	(94%)	(84%)	(93%)	(61%)	(97%)	(99.9%)	(99.4%)	(96%)
Trickling Filter Effluent	2.5	0.7	1.6	1.1	2.1	1.9	21	320	140	7.0
(% Reduction)	(73%)	(90%)	(95%)	(86%)	(71%)	(66%)	(70%)	(59%)	(85%)	(79%)
Flow (% of Mean Flow)	105	102	99	94	109	84	103	105	105	97
Mean Concentration Efficiency (%)	100	73	79	42	81	76	95	58	35	66

Uvalde--

Table 20 includes a summary of results of analyses for bacteriophage for most sampling points at the Uvalde site.

Bacteriophage concentrations found in the raw wastewater, like the total and fecal coliform concentrations, were somewhat below those observed at Kerrville. Net reductions throughout the treatment system appear to parallel those found for total and fecal coliform. The significance of the minor increase in bacteriophage concentrations in Cook's Slough is attributed to the impact of the wastewater treatment facility discharge.

Concentrations of enteroviruses observed in the raw wastewater were consistent despite the relatively dilute sewage (see Tables 24 and 25). The lack of removal of enteroviruses in the hydraulically overloaded trickling filter was anticipated (see Table 25). These results were in contrast with those obtained at the Kerrville site, where hydraulic loading is not a problem.

The observed increase in virus concentrations downstream of the site in Cook's Slough is consistent with the virus levels found in the effluent of Pond #6. Unfortunately, the virus isolates detected in Cook's Slough were not retained for confirmation and identification.

IRRIGATION POND STUDIES

Kerrville

A more detailed study of the Kerrville irrigation pond was undertaken because of the limited number of isolations of indigenous enteric viruses in irrigated effluent. As previous research in a model-pond system had documented viral deposition with particulate materials (Funderburg et al, 1978), a program of sediment sampling was initiated. Water column samples taken at approximately mid-depth and corresponding sediment samples were obtained on a line from the pond influent to the pond effluent as illustrated in Figure 9. Both water and sediment samples were analyzed for total and fecal coliforms, fecal streptococci, coliphage and enteric viruses.

Results presented in Table 26 show representative results from three separate sampling times. From an analysis of water column data collected in March and April, it appears that mixing within the pond was reasonably good. In general, variation in microbial concentrations at the three sampling points was insignificant. Levels of fecal streptococci and bacteriophage in pond effluent was lowest in August, perhaps due to higher summer temperatures and increased algal activity. During these special studies, no indigenous enteric viruses were recovered from 20-liter water samples concentrated at each point utilizing the Prototype 2 procedure as described in METHODS AND MATERIALS.

Not surprisingly, the levels of indicator organisms per equivalent unit (ml vs. gm) showed an enrichment of viable microorganisms in the

TABLE 24. Results of Wastewater Enterovirus Assay, Uvalde Site*

Sample Point	Range of Enterovirus Concentrations Recovered (pfu/l) 3-Day	Range of Enterovirus Concentrations Recovered (pfu/l) 5-Day	Remarks
Raw Wastewater	59-880	64-1340	
Trickling Filter Effluent	21-460	28-980	Chemical data indicate that trickling filter is biologically underloaded, though hydraulically overloaded.
Pond #1	12-240	12-860	
Pond #2	1.8-160	1.8-620	
Pond #3	6.0-172	7.6-355	Early in the study there was an indication that short circuiting of raw wastewater existed to Pond #3.
Pond #4	<1.1-97	6.5-257	
Pond #5	<1.1-21	<0.09-21	
Pond #6	<0.1=5.1	<0.09-]2	
Cook's Slough, Upstream	<0.5-4.6	<0.09-8.5	
Cook's Slough, Downstream	<0.5-1.5	<0.09-14	

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* Values derived from ten samples; in two cases grab samples were collected for all sample points.

TABLE 25. Effectiveness of Wastewater Treatment Processes in Reducing Enterovirus Recovery (Selected Sampling Days), Uvalde Site

Sample Point	Enterovirus Recovery(pfu/l, 5 day) and Cumulative Treatment Effectiveness (%)			
	Sampling Date			
	3/28-29/77	5/18-19/77	7/20-21/77	6/13-14/78
Raw Wastewater	102	341	1340	1100
Trickling Filter Effluent	80	340	805	980
(% Reduction)	(22%)	(.2%)	(40%)	(11%)
Pond #1 Effluent	63	93	415	860
(% Reduction)	(38%)	(73%)	(69%)	(22%)
Pond #4 Effluent	6.5	41	12	2.2
(% Reduction)	(94%)	(88%)	(99%)	(99.8%)
Pond #6 Effluent	1.1	1.2	<0.5	<0.28
(% Reduction)	(99%)	(99.6%)	(>99.96%)	(>99.97%)

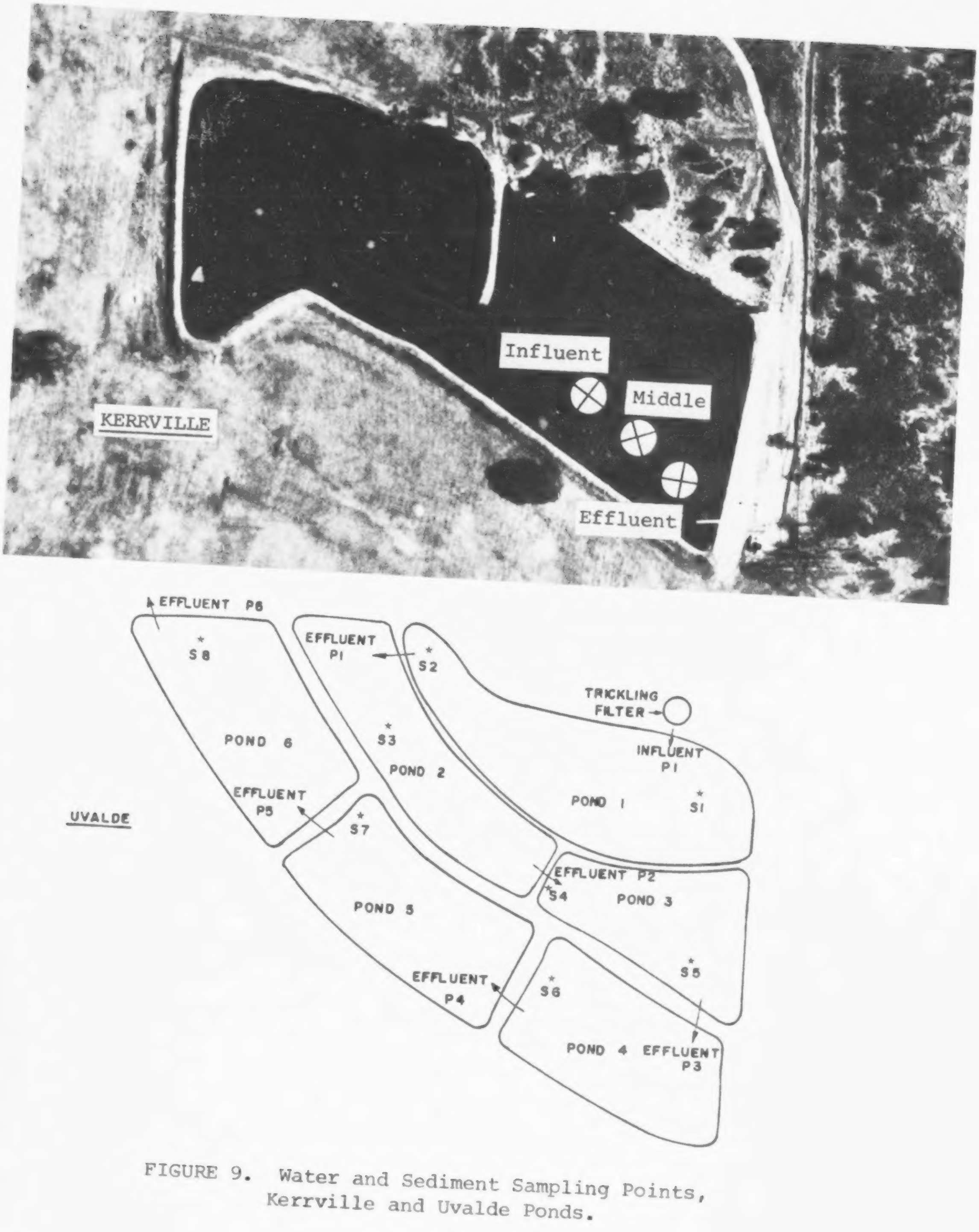
Influent
Middle
Effluent
KERRVILLE
EFFLUENT P6
S8
EFFLUENT P1
S2
TRICKLING FILTER
INFLUENT P1
POND 6
S3
POND 2
POND 1
S1
EFFLUENT P5
S7
UVALDE
EFFLUENT P2
S4
POND 3
POND 5
S5
EFFLUENT P4
S6
POND 4
EFFLUENT P3

FIGURE 9. Water and Sediment Sampling Points, Kerrville and Uvalde Ponds.

TABLE 26. Organism Distribution in the Kerrville Irrigation Pond

Sampling Point	Fecal Coliform (cfu/100 ml)	Fecal Streptococci (cfu/100 ml)	Bacteriophage[†] (pfu/l)
WATER COLUMN			
March, 1978			
Influent	$7.6x10^{3}$	$6.0x10^{2}$	$9.3x10^{4}$
Middle	$7.6x10^{3}$	$1.1x10^{3}$	$1.3x10^{5}$
Effluent	$3.6x10^{4}$	$3.0x10^{3}$	$8.5x10^{4}$
April, 1978			
Influent	$1.0x10^{5}$	$1.2x10^{3}$	$5.4x10^{4}$
Middle	$1.2x10^{5}$	$3.3x10^{3}$	$2.5x10^{4}$
Effluent	$7.0x10^{4}$	$3.7x10^{3}$	$5.8x10^{4}$
August, 1978			
Effluent	$1.7x10^{4}$	$2.7x10^{2}$	$2.0x10^{3}$
SEDIMENTS			
	(cfu/gm)	(cfu/gm)	(pfu/gm)
March, 1978*			
Influent	$2.8x10^{4}$	$9.7x10^{3}$	$4.3x10^{3}$
Middle	$1.4x10^{4}$	$2.5x10^{4}$	$3.6x10^{3}$
Effluent	$1.5x10^{3}$	$2.2x10^{4}$	$1.8x10^{2}$
April, 1978[α]			
Influent	$9.3x10^{3}$	$4.1x10^{5}$	$1.2x10^{3}$
Middle	$7.5x10^{4}$	$6.7x10^{5}$	$1.8x10^{4}$
Effluent	$2.8x10^{3}$	$5.7x10^{4}$	$4.2x10^{2}$
August, 1978*			
Influent	$1.1x10^{4}$	$2.3x10^{3}$	$5.0x10^{2}$
Effluent	$6.8x10^{3}$	$8.6x10^{3}$	$8.4x10^{2}$

† *E. coli* K13 as host organism.

α MPN procedure for bacterial indicators.

* Samples for bacterial analysis were spread plated onto appropriate selective media.

sediments. However, organism distribution at the sediment sampling points did not follow a consistent pattern. As indicated in Table 26, March values for fecal coliform and bacteriophage reflected an anticipated distribution with the highest concentrations detected at the influent sampling point where maximal sedimentation would be expected. On the other hand, fecal streptococci showed a relatively even distribution at all three sampling points. In April, the greatest concentration of all indicator organisms appeared at the middle sampling point. By August, little, if any difference in bacterial or coliphage densities could be ascertained between influent and effluent points. As with the water samples, fecal streptococci and bacteriophage levels in sediments were lowest in August.

The only positive enteric virus sediment sample obtained was taken in August at the effluent sampling point. Subsequent handling of the isolate resulted in its identification as a Coxsackievirus B5. It should be noted, however, that sample toxicity toward cell monolayers was a recurring problem in plating sediment concentrates.

Uvalde

In June, 1978, both pond waters and sediments were sampled at points indicated on Figure 9. As noted earlier, a reduction in microbial numbers was observed throughout the series of six ponds. Representative results showing fecal coliform and coliphage removals are presented graphically in Figure 10. An anomaly in the treatment effectiveness was noted in pond 3. Previous monitoring at the Uvalde site had suggested a possible source of infiltration into this pond. The aerial photograph presented as Figure 4 lends further credence to the probability of an inflow at the northeast corner of pond 3. In this picture, the visible turbidity throughout the third pond exceeded that present at the trickling filter outflow into pond 1. Nonetheless, the remaining ponds in series (4, 5 and 6) achieved bacterial reductions of three to four $\log_{10}$ as shown in Table 27. During the June sampling, fecal coliform levels, discharged to Cook's Slough, did not exceed 300 cfu/100 ml. In like fashion, coliphage concentrations in the final pond effluent were reduced to 400 pfu/liter. By concentrating a maximum of 20 liters of water, indigenous enteric viruses were detected only through pond 4 in the series.

An important mechanism of microbial removal during pond treatment appears to be deposition with particulate matter into bottom sediments. As shown in Table 27, the highest concentrations of all indicator organisms in pond sediments were observed at the influent end on pond 1. Additionally, higher numbers of total coliform, coliphage and enteric viruses were recovered from sediments located at the effluent end of pond 3. These findings further implicate an additional source of wastewater entering the third pond.

In general, sediment samples through pond 4 were enriched in the number of organisms recovered when compared to overlaying water. This phenomenon was particularly pronounced for fecal streptococci in ponds 1, 2, 3 and 4 and suggests prolonged survival of these enteric organisms

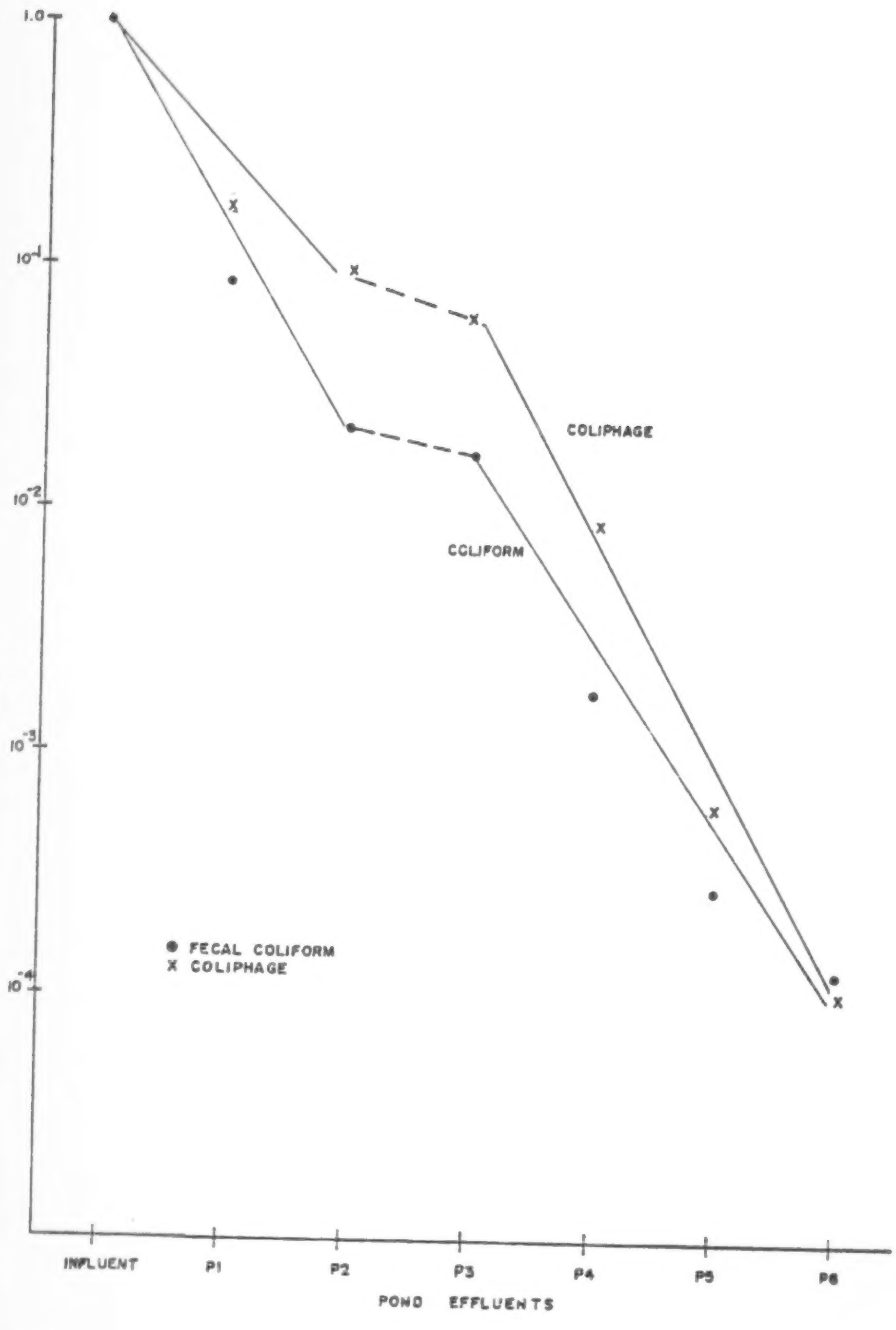

FIGURE 10. Fecal Coliform and Coliphage Removals in Uvalde Ponds

TABLE 27. Microbial Profiles in Uvalde Ponds (June, 1978)

Sampling Point	Total Coliforms		Fecal Coliforms		Fecal Streptococci		Bacteriophage[α]		Enteric Viruses	
	Water (cfu/100 ml)	Sediment (MPN/gm)	Water (cfu/100 ml)	Sediment (MPN/gm)	Water (cfu/100 ml)	Sediment (MPN/gm)	Water (pfu/l)	Sediment (pfu/gm)	Water (pfu/l)[β]	Sediment[γ] Total pfu Confirmed
Pond 1										
Influent	$2.1x10^{7}$	$1.5x10^{6}$	$2.2x10^{6}$	$9.4x10^{4}$	$8.7x10^{4}$	$1.7x10^{5}$	$3.5x10^{6}$	$1.1x10^{6}$	$5.0x10^{2}$	28
Effluent	$1.5x10^{6}$	$8.7x10^{4}$	$1.9x10^{5}$	$8.7x10^{4}$	$1.7x10^{3}$	$1.1x10^{5}$	$6.4x10^{5}$	$2.1x10^{4}$	$4.4x10^{2}$	5
Pond 2										
Middle		$6.0x10^{5}$		$2.0x10^{5}$		$2.3x10^{5}$		$6.0x10^{4}$		1
Pond 3										
Influent	$3.4x10^{5}$	$1.9x10^{5}$	$4.8x10^{4}$	$1.8x10^{5}$	$9.7x10^{2}$	$1.2x10^{4}$	$3.4x10^{5}$	$4.2x10^{4}$	$3.2x10^{2}$	2
Effluent	$3.1x10^{5}$	$1.4x10^{6}$	$3.8x10^{4}$	$2.2x10^{5}$	$2.9x10^{2}$	$4.0x10^{4}$	$2.2x10^{5}$	$2.8x10^{5}$	$1.6x10^{1}$	6
Pond 4										
Effluent	$2.4x10^{5}$	$5.8x10^{5}$	$3.9x10^{3}$	$4.1x10^{5}$	$4.0x10^{1}$	$1.7x10^{3}$	$3.1x10^{4}$	$5.4x10^{4}$	$8.0x10^{-1}$	ND
Pond 5										
Effluent	$3.1x10^{4}$	$3.6x10^{2}$	$6.1x10^{2}$	$<3.6x10^{2}$	$1.7x10^{1}$	$<3.6x10^{2}$	$2.2x10^{3}$	$<3.7x10^{1}$	ND	ND
Pond 6										
Effluent	$6.1x10^{3}$	$2.3x10^{3}$	$2.9x10^{2}$	$<1.4x10^{2}$	$3.7x10^{1}$	$5.1x10^{2}$	$4.0x10^{2}$	$<2.7x10^{1}$	ND	ND

ND None Detected

[α] Direct plate of sample on *E. coli* K13

[β] pfu on HeLa and BGM cells; corrected for concentration efficiencies ($\bar{x}$ = 50%)

[γ] Sediment elution with 3% beef extract; reconcentration by organic flocculation; assay on HeLa cells.

in pond sediments. While this study was limited in that no attempts were made to measure quantitatively the amount of solids deposited in the ponds, the number of viable organisms in the sediment sink, through pond 4, could constitute a large microbial reservoir.

IRRIGATION AND PRECIPITATION

Table 28 contains a summary of wastewater flow, irrigation and precipitation data for the period approximating the final year of the study. Wastewater flow over the period was relatively stable except immediately after major periods of precipitation (note 10.4 inches of precipitation during the period July 24-August 1, 1978), which resulted in significant infiltration. Therefore, wastewater available for irrigation was relatively constant ranging from 1.7 to 4.7 inches/week. More often than not, however, it was close to the mean rate of 2.7 inches/week. Actual irrigation rates ranged from zero to 4.2 inches/week with a mean of 2.4 inches/week. This is a direct result of the operation of an irrigation system as a wastewater treatment (control) process with interest in irrigation being secondary. This is not a negative statement but rather a fact of life. Under these circumstances, irrigation is practiced when wastewater begins to accumulate normally during periods of high precipitation and low evaporation. As indicated in Table 28, the highest irrigation rates occurred in November 1977, following significant precipitation (5.2 inches in less than three weeks).

SOIL ANALYSES

Chemical Properties

Table 29 contains the results of selected chemical and physical analyses of surface soil samples. The pH analyses of the soils at the Kerrville and Uvalde sites indicate that these are intermediate to basic range soils. Relatively high soil pH is to be expected in light of the basic calcium carbonate parent rock in the area. The cation exchange capacity (CEC) of the soils from both sites is in the medium to high range, again indicating similar soil material and, therefore, similar exchange capacity. The organic content of the soils from the two sites differs substantially, however. The Uvalde soils fall within the normal range in organic content, while the Kerrville soils are somewhat high. The higher organic content of some of the soils at the Kerrville site is related primarily to the long-term use of the site for irrigation by the application of wastewater.

Soil samples were collected from the upper 0.2M of the soil profile from each major soil series present at the Kerrville site. The soil samples were analyzed for Pb, Cd, Fe, Cr, Cu, Mn, Ni, Na, K, Ca, Mg and Zn. Results of these analyses are shown in Table 30. The metallic ion concentration in a soil is the result of a complex series of geochemical reactions involving mineral weathering and decomposition, plant activity and animal activity. The total concentrations of metallic ions in soils usually relates to the concentrations of the metallic ions present in

TABLE 28. Application of Wastewater and Precipitation (October 1977-August 1978) - Kerrville Site

Year	Sampling Period	Wastewater Flow Rate (MG/week)	Weeks in Period	Wastewater Available for Irrigation (in/week)	Wastewater Irrigation Rate (in/week)	Precipitation Rate (in/week)	Gross Irrigation Rate (in/week)
1977	10/13-10/19	11.0	1.00	3.0	3.7	0	3.7
	10/20-10/26	8.0	1.00	2.2	1.9	3.2	5.1
	10/27-11/2	9.7	1.00	2.7	0.1	0.3	0.4
	11/3-11/9	9.3	1.00	2.6	4.2	1.7	5.9
	11/10-11/16	10.8	1.00	3.0	3.0	0	3.0
	11/17-12/7	9.0	3.00	2.5	1.1	0.4	1.5
	12/8-1/24	9.2	6.86	2.5	2.2	0.1	2.3
1978	1/25-2/3	10.4	1.43	2.9	1.9	0.3	2.2
	2/4-2/17	12.0	2.00	3.3	2.0	1.0	3.0
	2/18-2/22	10.7	0.71	3.0	2.9	0.7	3.6
	2/23-3/7	10.1	1.86	2.8	2.0	0.3	2.3
	3/8-3/28	10.4	3.00	2.9	2.0	0	2.0
	3/29-4/11	8.9	2.14	2.5	1.9	0.8	2.7
	4/12-5/3	8.6	3.14	2.4	2.0	0.6	2.6
	5/4-5/22	9.0	2.71	2.5	2.0	0.7	2.7
	5/23-5/31	8.0	1.28	2.2	2.0	0.5	2.5
	6/1-6/20	12.6	2.86	3.5	2.1	1.6	3.7
	6/21-7/10	6.2	2.86	1.7	2.7	0	2.7
	7/11-7/24	8.9	1.86	2.5	1.9	0.2	2.1
	7/24-8/1	10.8	1.28	3.0	1.4	10.4	11.8
	8/2-8/9	16.4	1.14	4.5	0.4	0.6	1.0
	8/10-8/16	16.9	1.00	4.7	2.9	0	2.9
Means for Entire Period		9.9	-	2.7	2.4	0.8	3.2

TABLE 29. Selected Properties of Surface Soil Samples

Soil Sample Location*	Moisture (%)	pH (Units)	CEC (meq/10w gm)	Organic Content (% Dry Weight)
Kerrville:				
#1	14-34	7.9	24-43	2.7-3.9
#2	17-19	7.8-8.0	19-48	1.7-3.1
#3	3-26	7.7-8.3	27-31	2.1-5.3
#4	5-23	8.1-8.6	20-21	1.3-3.3
#5	20-29	7.7-8.2	24-36	2.4-3.4
#6	26-28	7.8-8.0	18-29	1.6-3.2
Uvalde:				
#1	18	8.0	32	1.6
#2	17	8.0	27	1.5
#3	12	7.7	24	2.1
#4	15	7.9	25	1.8

* Samples collected on October 29, 1976 and March 29, 1978 at Kerrville and on March 4, 1977 at Uvalde.

TABLE 30. Soil Metallic Ion Concentrations (µg/5 grams)

SOIL SERIES	Cd	Zn	Mn	Pb	Cu	Ni	Cr	Fe	Mg	Ca	K	Na
Frio	< 0.03	.54	25.83	1.00	.28	.28	.81	34	200	18,2[illegible]0	185	5.25
Denton (1) Moderate Slope	< 0.03	.16	18.90	.63	.31	.42	.80	60	225	21,300	117	10.00
Denton (2) Level	0.05	4.83	26.88	2.50	1.10	.42	.86	88	525	17,000	147	6.25
Lewisville	< 0.03	.55	15.75	1.00	.59	.36	.68	147	513	17,200	122	31.25
Brackett	< 0.03	.31	19.11	.88	.47	.30	.68	60	150	18,530	129	3.75

the parent materials. The high calcium content (17,000-21,000 μg/5 grams) of the Kerrville site soils reflects the parent calcium carbonate bedrock.

The metallic ion concentrations in the Kerrville site soils, in particular those soils in the upland areas (Brackett series) which have not been subjected to intensive irrigation with wastewater, are probably representative of the natural soil metallic ion concentrations prior to irrigation. The soil samples from the lowland, level Denton soil series show an increase in cadmium, zinc and copper concentrations when compared with the concentration present in the other soil series. This increase in these ions indicates the more intense utilization of these lowland sites for wastewater application.

Mineralogy

Silt Size Range--

Table 31 shows the analyses of the silt size range for each major soil type at the Kerrville site. Minerals present in the silt size range of the soil samples were calcite ($CaCO_5$), quartz (SiO_2) and gypsum ($CaSO_4$) as determined by X-ray diffraction. These minerals are the result of weathering and erosion of the Cretaceous carbonate strata in the drainage basin of Third Creek.

TABLE 31. Minerals Present in the Silt Size Range

Dominant Mineral Present Listed First

Denton Series	Lewisville Series
Calcite	Calcite
Quartz	Gypsum
Gypsum	Quartz
Frio Series	Brackett Series
Quartz	Quartz
Calcite	Calcite
Gypsum	Gypsum

Clay Size Range--

Table 32 lists the mineral content of clay size particles present in the soils at the Kerrville site. The minerals present include montmorillite, kaolinite, calcite, quartz and gypsum.

Montmorillite is a term used to describe a group of hydrated silicates with the general formula $(OH)_4Si_8Al_4O_{20}.xH_2O$ where x molecules of water can be driven off at low temperatures. Montmorillite is composed of a single sheet of silica tetrahedrons arranged as octahedrons enclosed by two silica tetrahedral sheets (Figure 11). The oxygen atom layers at the base of the tetrahedron are adjacent to each other, causing a weak bond and facilitating excellent cleavage. The tetrahedron layers carry a negative charge on their surfaces which attracts varying amounts of water. Calcium and sodium cations can enter the layered silicate structure and there is some substitution within the layers. Montmorillite is therefore a clay mineral with a high cation exchange capacity.

Kaolinite is a clay mineral composed of a single octahedral sheet and a single tetrahedron sheet. The sheets of octahedrons and tetrahedrons are combined or stacked and tied together with hydrogen bonds. The formula of kaolinite is $(OH)_8Si_4Al_4O_{10}$. The atomic arrangement of kaolinite allows little substitution within the kaolinite lattice and kaolinite has a limited cation exchange capacity (Figure 11). Because of the relatively weak hydrogen bond, kaolinite can be split into very thin platelets which are negatively charged on their flat surfaces and will attract thick layers of water. These water layers in kaolinite produce the characteristic plasticity exhibited when kaolinite is mixed with water.

TABLE 32. Minerals Present in the Clay Size Fraction; Dominant Mineral Present Listed First

Denton Series	Lewisville Series	Frio Series	Brackett Series
Montmorillite	Montmorillite	Montmorillite	Montmorillite
Kaolinite	Kaolinite	Kaolinite	Kaolinite
Calcite	Calcite	Calcite	
	Gypsum	Quartz	
	Quartz	Gypsum	

Soil Particle Size Distribution

The size distribution of the soil particles at the Kerrville site

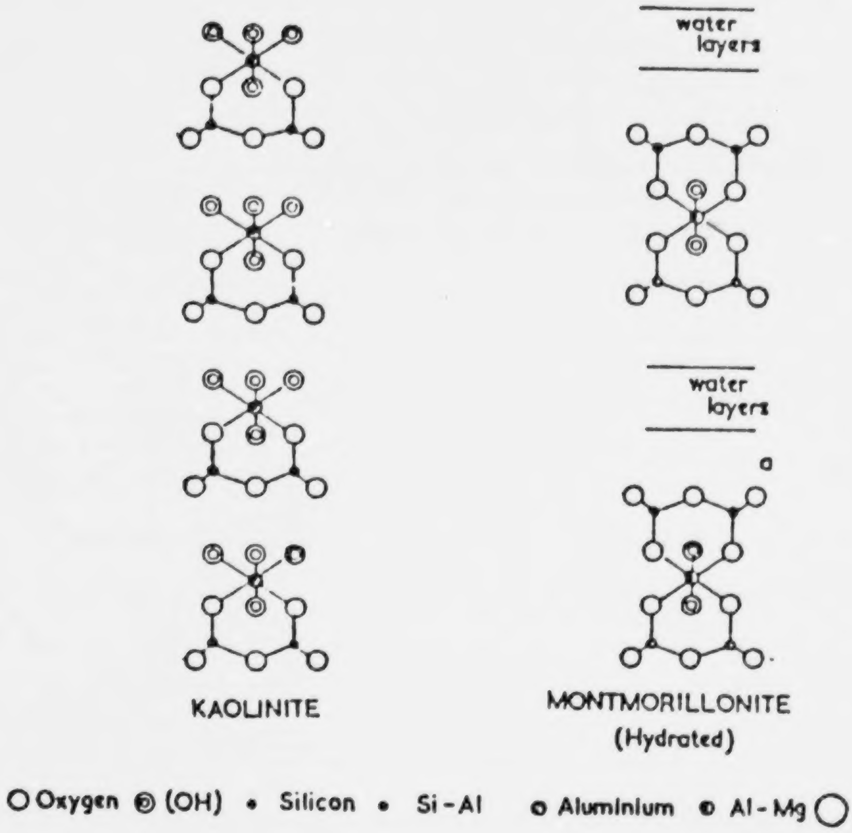
water layers
water layers
KAOLINITE
MONTMORILLONITE
(Hydrated)
○ Oxygen
◎ (OH)
• Silicon
• Si-Al
⊙ Aluminium
⊙ Al-Mg ○

FIGURE 11. Diagrammatic Representation of the Succession of Layers in Some Layer Lattice Silicates. (Brown, 1961)

is important in the determination of the dimensions of available pore space in the soils. The size of the pores determines the movement and distribution of water and gases in the soil. Soil aggregate size distribution will vary in a given irrigation site dependent on the amount of water applied and duration of irrigation. Figure 12 presents the soil aggregate distribution curves for soil samples from the Kerrville site.

The clay minerals in the soils at the Kerrville site are predominantly montmorillonitic. These soils have a cation exchange capacity (CEC) ranging from 1.7 to 67 meq/100 gm. While not all of the CEC is directly attributable to the type and quantity of clay mineral present in a given soil, the predominance of one clay mineral over another will determine the suitability of the soil as a wastewater renovation site.

Microbiological Assays

Table 33 illustrates the ranges of both total and fecal coliforms enumerated in soils taken from the Kerrville site over a 16-month period from October, 1976 through February, 1978. During the early stages of this study, sample points 1 through 4 received considerably less wastewater irrigation than did sampling points 5 and 6. Thus, variability was observed in the early results, although differences lessened as irrigation practices became more balanced over the site.

The fact that less irrigation was done in area 1 over the sanitary landfill was reflected by overall lower levels of fecal coliform at soil site number 4. Additionally, only one soil sample from this location yielded a positive isolation of coliphage. In all other irrigated areas, fecal coliform counts ranged as high as 10^4-10^6 MPN/gm of dry soil. In addition, multiple numbers of samples were positive for bacteriophage with levels ranging maximally to 5 pfu/gm and 16 pfu/gm.

Viewed on a monthly basis, the levels of both total and fecal coliform isolated from the soils at Kerrville correlates reasonably well with irrigation practices. For example, it is the practice of operating personnel to irrigate more during periods of rainfall regardless of season; less during dry cool periods and even less during dry hot periods. This operating procedure insures maximal capacity in the ponds and precludes direct discharge to Third Creek. The field data reflect these conditions as the lowest values of both total and fecal coliform generally were observed in December and again in July and August while the highest values were recorded in May, June, September and October. Table 34 illustrates this observation more conveniently. High total coliform to fecal coliform ratios were indicative of low irrigation periods.

Because of the overriding influence of irrigation at the Kerrville site, no conclusions could be reached regarding organism survival as influenced by seasonal conditions. It is interesting to note, however, that bacteriophage were isolated from soils in all seasons.

In order to observe more directly the effect of irrigation on indicator organism levels in soil, two special studies were undertaken.

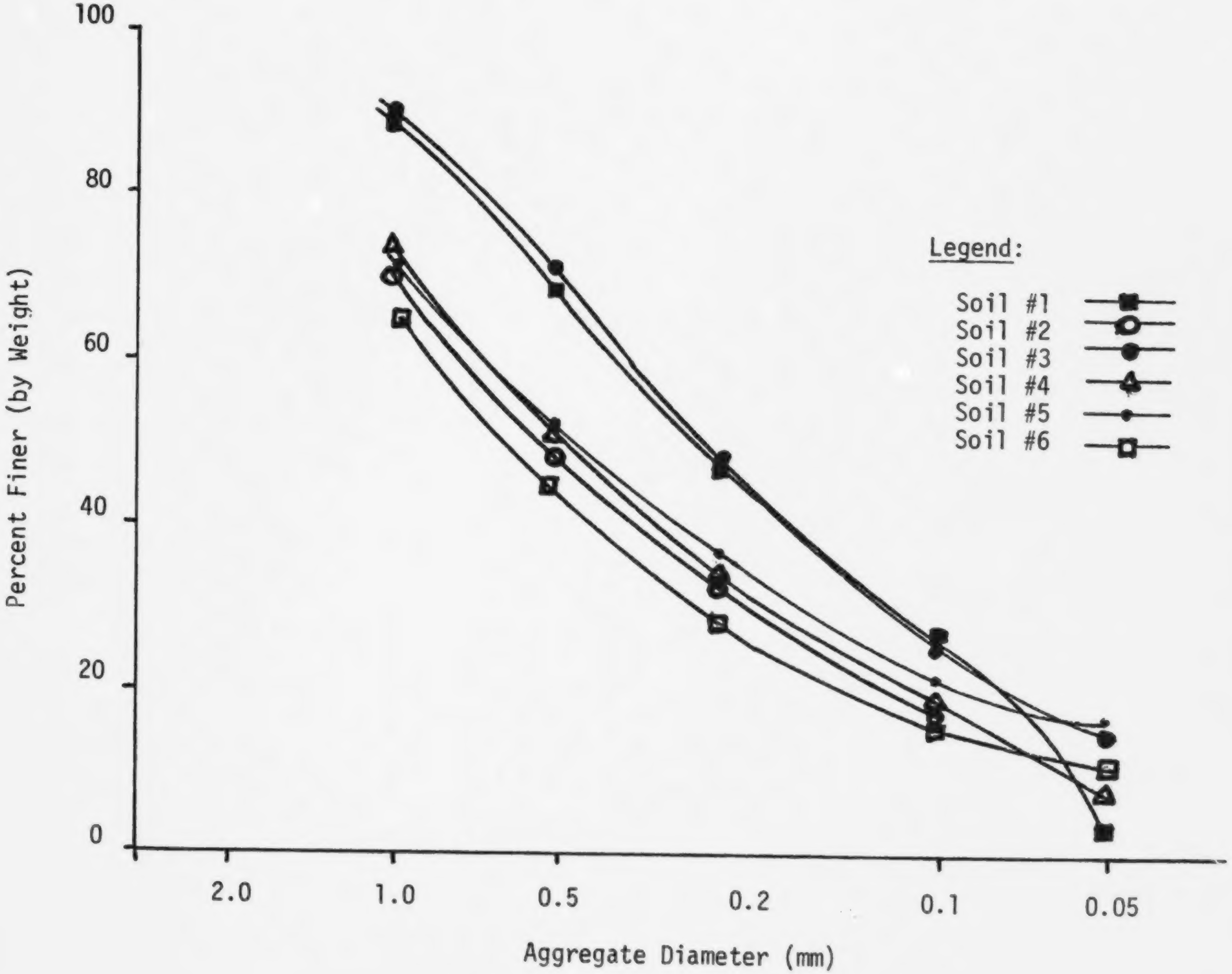

FIGURE 12. Soil Aggregate Distribution Curves, Kerrville Soils (Surface Samples)

TABLE 33. Ranges of Results of Soil Bacteria and Bacteriophage Analysis, Kerrville Site*

Soil Sample Location +	Total Coliform (MPN/gm)	Fecal Coliform (MPN/gm)	Bacteriophage (pfu /gm)	Remarks
#1	$6.0 \times 10^{1} - 3.7 \times 10^{5}$	$4.0 \times 10^{0} - 3.6 \times 10^{5}$	< 0.1 - 0.8	3 positive bacteriophage samples
#2	$1.8 \times 10^{1} - 6.4 \times 10^{6}$	$4.5 \times 10^{-2} - 6.4 \times 10^{6}$	< 0.1 - 2.9	8 positive bacteriophage samples
#3	$1.1 \times 10^{1} - 4.2 \times 10^{5}$	$4.4 \times 10^{-2} - 6.7 \times 10^{4}$	< 0.1 - 0.9	4 positive bacteriophage samples
#4	$< 3.9 \times 10^{0} - 9.1 \times 10^{5}$	$4.3 \times 10^{-2} - 5.2 \times 10^{3}$	< 0.1 - 4.8	1 positive bacteriophage sample
#5	$1.2 \times 10^{1} - 5.5 \times 10^{5}$	$4.7 \times 10^{-2} - 3.9 \times 10^{4}$	< 0.1 - 16.0	3 positive bacteriophage samples
#6	$1.9 \times 10^{1} - 2.6 \times 10^{5}$	$2.6 \times 10^{0} - 1.6 \times 10^{4}$	< 0.1 - 3.8	4 positive bacteriophage samples

* Values Derived from 16 Sampling Times over 16-Month Period

+ See Figure 2 for Sampling Point Locations

During January and February, 1978, and again in May, 1978, arrangements were made with local personnel to manage the areas around selected lysimeters over a 10-day study period.

Microbial levels observed during the first study in soil sampling area 2 are presented in Table 35. Enumeration of indicator organisms in grab samples taken at the irrigation pond outlet during spraying were quite constant with mean values of 1.3×10^6 total coliform/100 ml, 4.3×10^5 fecal coliform/100 ml, and 5.4×10^3 fecal streptococci/100 ml. Soil samples were taken approximately 18 hours after cessation of wastewater application. While the levels of total coliform show an increase relative to the controls during sustained irrigation, even greater rises in the levels of both fecal coliforms and fecal streptococci were observed in irrigated soils as compared to the non-irrigated controls. Bacteriophages plaquing on *Escherichia coli* K13 were observed at levels ranging from 0.2 pfu/gm to $> 10^2$ pfu/gm by directly plating soils eluates. Using an elution-reconcentration scheme for soil samples as described previously, any areas showing cytopathic effects on either HeLa or BGM monolayers were picked as potential viral isolates. However, subsequent tube passage failed to confirm viral isolates.

The second controlled irrigation study was conducted in the area around lysimeter 2. Results shown in Table 36 reflect similar bacterial trends. Bacteriophage consistently were recovered from irrigated soils at levels ranging from 1.0 pfu/gm to 4.5 pfu/gm of soil. During this study, a single human enteric virus, subsequently identified as poliovirus 1, was recovered from an irrigated soil.

It should be noted that only one other enteric virus (also poliovirus 1) was isolated from soil samples over the two-year study period. While as many as 150 potential viral isolates (as defined by any evidence of cpe) have been picked to tube cultures, only two could be confirmed. The prevalence of soil microorganisms capable of cell destruction mimicking viral cpe has been noted previously in samples from sludge-injected soils and serves to emphasize the importance of viral confirmation procedures.

Data summarizing the soil analyses conducted at the Uvalde site are presented in Table 37. In comparison to the Kerrville data, it is obvious that the levels of both bacterial indicators and coliphage are significantly lower (several orders of magnitude). This, in part, may be attributed to the less structured irrigation schedule at Uvalde. It is interesting to note, however, that a similar pattern exists for the total coliform to fecal coliform ratios. As can be seen in Table 38, these ratios are in the same range as those at the Kerrville site during periods of more intense irrigation.

LYSIMETERS

Soil Particle Size Analysis

The subsurface soils at the lysimeter sited are composed predominately

TABLE 34. Ratio of Soil Total Coliform to Fecal Coliform, Kerrville Site

Sample Point location	Sampling Date 10/27/76	11/23/76	12/16/76	1/13/77	2/24/77	3/17/77	4/30/77	5/27/77	6/15/77	6/30/77	7/18/77	8/3/77	9/28/77	10/26/77	11/28/77	2/21/78
#1	*	11	> 220	1	> 7	1	10	36	70	> 35	160	23	*	> 2	1	30
#2	11	400	> 310	> 12	260	8	> 27	7	1	55	140	17	11	5	2	5
#3	48	250	> 4700	13	> 12	5	30	90	6	180	74	> 122	8	> 9	1	> 6
#4	1	170	2300	8	> 120	*	> 9	13	18	3	> 180	> 85	2	> 4	2	2
#5	2	260	> 85	59	> 16	15	3	14	5	> 85	> 1200	109	*	3	> 6	29
#6	2	7	190	50	15	> 100	> 170	42	16	160	300	226	*	> 4	7	61

* Ratios were not calculable.

TABLE 35. Soil Data, Special Irrigation Study I, Kerrville Site ≠

	1-27-78 (α)			1-30-78			2-1-78			2-3-78		
	Irrigated		Control	Irrigated		Controlβ	Irrigated		Control	Irrigated		Control
Parameter	S1	S2		S1	S2		S1	S2		S1	S2	
Moisture (% water)	-	-	-	29	19	15	27	25	17	28	23	18
Total Coliform (MPN/g dry wt)	$3.9x10^{4}$	$2.1x10^{5}$	$3.3x10^{4}$	$4.3x10^{4}$	$6.4x10^{4}$	$7.2x10^{3}$	$3.1x10^{4}$	$5.7x10^{3}$	$8.9x10^{2}$	$2.6x10^{4}$	$1.1x10^{4}$	$3.7x10^{2}$
Fecal Coliform (MPN/g dry wt)	$1.1x10^{3}$	$1.1x10^{3}$	$1.5x10^{2}$	$8.2x10^{3}$	$1.8x10^{3}$	$3.6x10^{2}$	$5.7x10^{3}$	$3.0x10^{3}$	$4.4x10^{1}$	$8.1x10^{3}$	$2.5x10^{3}$	$3.6x10^{1}$
Fecal Streptococci (MPN/g dry wt)	$5.0x10^{1}$	$1.8x10^{1}$	$1.1x10^{0}$	$8.2x10^{1}$	$3.6x10^{2}$	$1.1x10^{0}$	$1.3x10^{2}$	$7.5x10^{1}$	$6.2x10^{1}$	$1.3x10^{3}$	$1.2x10^{2}$	$3.6x10^{0}$
Bacteriophage (pfu/g dry wt)	$1.1x10^{0}$	$1.1x10^{0}$	< 0.1	$1.8x10^{2}$	$1.8x10^{2}$	$2.4x10^{2}$	$3.2x10^{0}$	$6.2x10^{0}$	$5.0x10^{-1}$	$2.5x10^{0}$	$2.0x10^{-1}$	$2.0x10^{-1}$
Enteric Viruses (pfu/g dry wt)	-	-	-	$< 5.0x10^{-3}$	$< 5.0x10^{-3}$	$< 5.0x10^{-3}$	$< 5.0x10^{-3}$	$< 5.0x10^{-3}$	$< 5.0x10^{-3}$	$< 5.0x10^{-3}$	$< 5.0x10^{-3}$	$< 5.0x10^{-3}$

≠ Wastewater applied: 1-29-78, 7.2 inches; 1-31-78, 10.8 inches; 2-2-78, 6.6 inches.

α Last previous irrigation, 1-24-78.

β Control soil collection site was observed to be inside spray wet line due to high winds, therefore non-irrigated soil site was moved further out for subsequent sampling.

TABLE 36. Soil Data, Special Irrigation Study II
Kerrville, Texas♦

Parameter	4-28-78α			5-2-78			5-4-78		
	Irrigated		Control	Irrigated		Control$^{\beta}$	Irrigated		Control
	S1	S2		S1	S2		S1	S2	
Moisture (% Water)	16	14	20	36	32	32	32	34	28
Total Coliform (MPN/g dry wt)	2.0×10^{1}	4.5×10^{2}	4.9×10^{1}	4.5×10^{4}	4.2×10^{5}	1.9×10^{3}	5.9×10^{3}	5.8×10^{4}	4.0×10^{1}
Fecal Coliform (MPN/g dry wt)	$<6.8 \times 10^{0}$	$<4.1 \times 10^{0}$	$<7.8 \times 10^{0}$	3.4×10^{2}	4.5×10^{3}	8.6×10^{2}	3.4×10^{3}	5.1×10^{2}	3.0×10^{1}
Fecal Streptococci (MPN/g dry wt)	$<6.8 \times 10^{0}$	$<4.1 \times 10^{-1}$	$<7.8 \times 10^{0}$	7.1×10^{1}	4.5×10^{2}	8.6×10^{1}	2.4×10^{3}	8.9×10^{2}	2.2×10^{3}
Bacteriophage (pfu/g dry wt)	<0.1	< 0.1	< 0.1	4.5	1.3	0.2	4.4	2.1	< 0.2
Enteric Viruses (pfu/g dry wt)	---	---	---	< 0.1	0.1 (1-polio 1)	< 0.2	< 0.2	< 0.2	< 0.2

♦ Cumulative Wastewater Application $\geq$ 18 inches, 1.9 inches rainfall recorded

α Pre-Irrigation

β Rainfall Recorded

TABLE 37. Ranges of Results of Soil Bacteria and Bacteriophage Assays, Uvalde Site*

Soil Sample Location	Total Coliform (MPN/gm)	Fecal Coliform (MPN/gm)	Bacteriophage (pfu/gm)	Remarks
#1	$2.2\times10^{1}-1.2\times10^{6}$	$9.1\times10^{-1}-4.8\times10^{4}$	$< 0.1-0.08$	2 positive bacteriophage samples
#2	$3.7\times10^{0}-1.9\times10^{4}$	$4.3\times10^{-2}-7.4\times10^{3}$	$< 0.1-0.49$	2 positive bacteriophage samples
#3	$2.7\times10^{2}-5.5\times10^{4}$	$4.2\times10^{-2}-3.3\times10^{4}$	$< 0.1-0.49$	1 positive bacteriophage sample
#4	$1.6\times10^{1}->5.3\times10^{4}$	$8.8\times10^{0}- 5.3\times10^{4}$	$< .1<0.49$	

* Values Derived from 5 Samples.

TABLE 38. Ratio of Soil Total Coliform to Fecal Coliform, Uvalde Site

Sample Point Location	Sampling Date				
	12/2/76	3/4/77*	5/20/77	7/20/77	11/2/77
#1	180	> 5	3	3	25
#2	86	> 6	3	12	14
#3	†	> 110	> 2	> 66	> 122
#4	43	> 270	α	2	10

* All fecal coliform values below analytical sensitivity.

† Calculated ratio of $1.7x10^5$.

α Both values too high for greatest dilutions; ratio incalculable.

of silt size particles (0.0625 mm to 0.0039 mm). The soil samples ranged from 90% to less than 20% silt size particles. Clay size fraction (diameter less than 0.0039 mm) ranged from 30% to less than 1%.

The coarse fraction of sand (2.00 mm to 0.0625 mm) and granule size particles (4 mm to 2 mm) were composed entirely of rounded limestone fragments and some cemented $CaCO_3$ concretions. The coarse fraction in the lysimeter soils ranged from 80% to less than 10% of the total soil sample.

The particle size distribution curves for the lysimeter sites (Figures 13-15) at depths of 1.5 ft, 3.0 ft and 4.5 ft illustrate the general pattern of soil particle size distribution. They provide more detail regarding the sand, silt and clay size fraction distribution for each of the lysimeter sites.

The vertical distribution of grain size at lysimeter sites #1 and #3 indicates a much lower clay size component in samples from the 4.5 foot depth, with increasing clay size content in the upper soil layers. The increased clay content in the upper soil layers is a result both of maximum mineral weathering at or near the surface and of an influx of alluvial clay materials. (Note, lysimeter site #2 does not follow this pattern; see Fig.14)

The silty clay and loam soils of the Kerrville site have a controlling effect on the suitability of the site for wastewater renovation. As soil particle size decreases, there is an exponential increase in soil adsorption capacity and surface area. The soil grain size distribution is important in determining the soil permeability and infiltration rate, also. The fine-grained soils at the Kerrville site are classified as having moderate permeability, ranging from 0.6 to 2.0 inches per hour. It should be noted that the actual characteristics of the soils at the lysimeter sites differ from those described for this general geographical area.

Chemical Properties of the Soil

Soil chemistry investigations at the Kerrville site involved the determination of pH, organic carbon, and cation exchange capacity. Results are shown in Table 39.

Soil pH--

The soil pH depends upon the amount and type of exchangeable ions present in the soil. The soil pH can also be changed by biological activity, by introduction of organic matter and, to a slight degree by changes in water content. The pH of the soils at the lysimeter sites ranged from 8.8 to 9.2 indicating a high concentration of the two principal bases, Ca^{2+} and Mg^{2+}. The soil pH was not observed to vary greatly with depth, indicating that the application of wastewater (pH of 7.5-8.9) had not significantly reduced the soil pH.

Organic Carbon--

Organic carbon in the Kerrville site soils ranged from 0.19 to 0.97% with a general trend toward an increase in organic carbon content in the upper soil layer at the lysimeter sites.

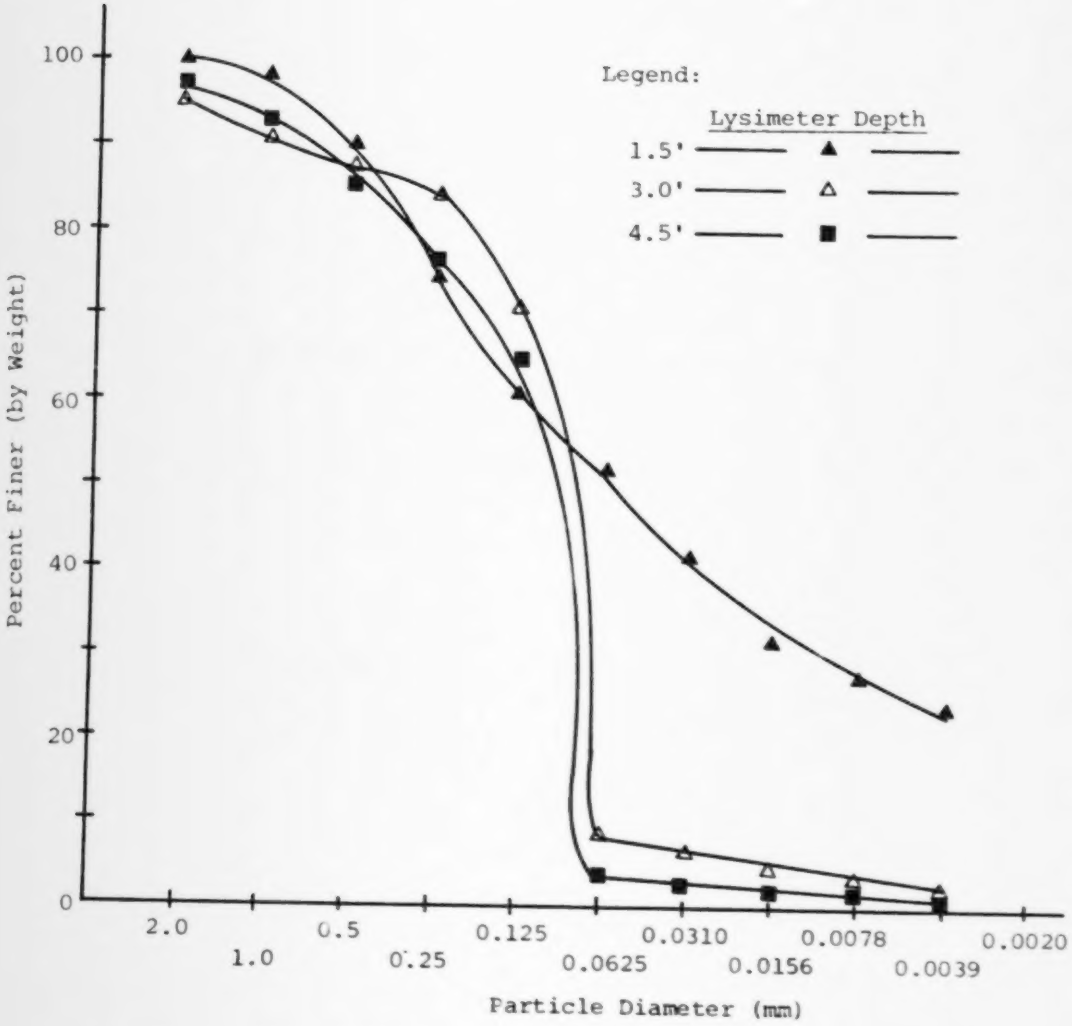

FIGURE 13. Soil Particle Size Distribution Curves for Lysimeter Site #1

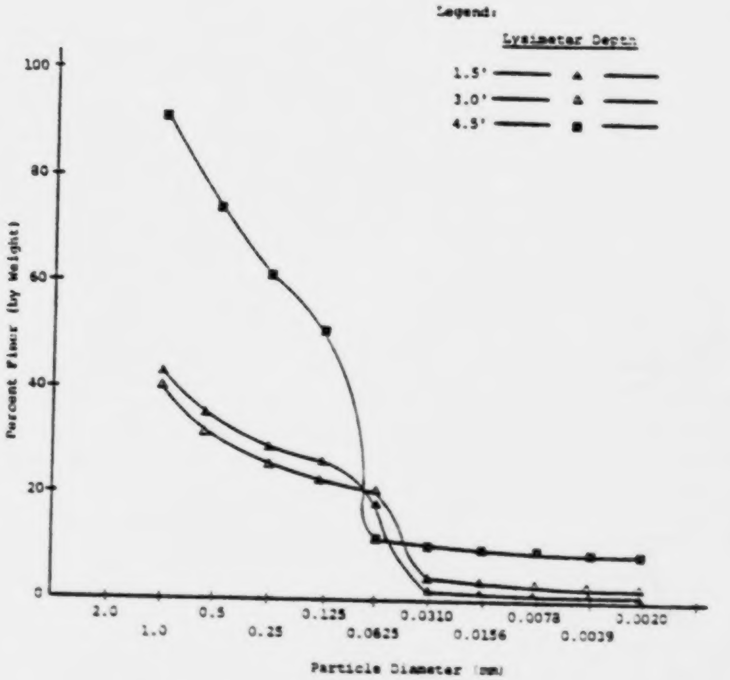

FIGURE 14. Soil Particle Size Distribution Curves: Lysimeter Site #2

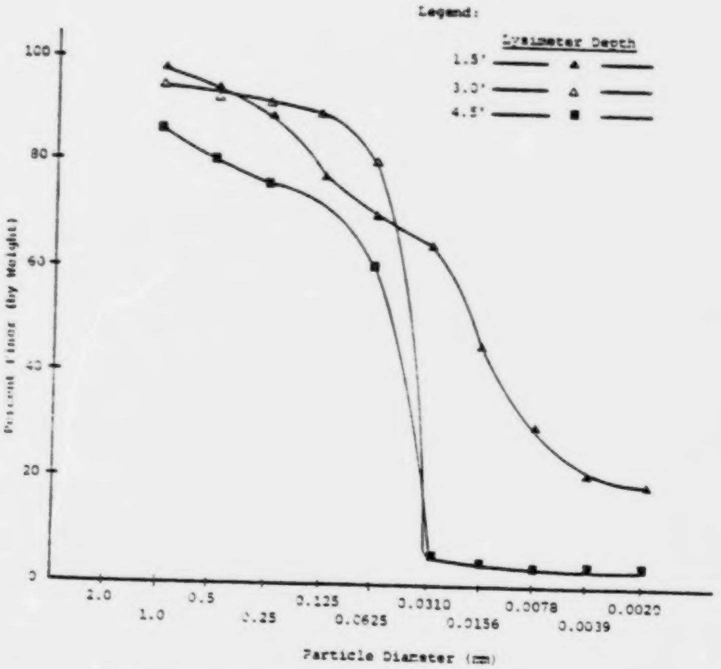

FIGURE 15. Soil Particle Size Distribution Curves: Lysimeter Site #3

Carbon is the chief component of soil organic matter and is derived from plant and animal remains and decayed organic matter. The amount of soil organic material plays an important role in the exchange capacity of a soil, especially at high pH values. In addition, the soil organic material has an effect on soil moisture retention and pH and in maintaining soil structure. Long-term application of wastewater would tend to increase the organic carbon content of the soil unless there was a corresponding increase in soil microbial activity to utilize the additional carbon effectively.

Cation Exchange Capacity--

The cation exchange capacity (CEC) of a soil indicates the quantity of ions held in an exchangeable form. Generally, CEC is highest for organic matter, is intermediate for the expanding clay minerals and is lowest in the clays with low expansion coefficients. The primary controlling factors in soil CEC are mineral content and the amount of organic material present. Additional factors contributing to the CEC are pH and the size of the soil material present. CEC values in the Kerrville site soils ranged from 21.2 meq/100 gm to 89.2 meq/100 gm. The CEC generally increased with depth (Table 39).

The decrease in CEC in the upper layers, in spite of a higher clay size fraction content, may be attributed to depletion of ion concentration by leaching of water percolating through the soil.

TABLE 39. Chemistry - Kerrville Lysimeter Soils

LYSIMETER	DEPTH (FT.)	pH	ORGANIC CARBON (%)	CATION EXCHANGE CAPACITY (meq/100 gm)
1	1.5	8.96±.25	.27±.17	21.2±.7
	3.0	8.89±.22	.38±.07	67.5±3.4
	4.5	8.82±.13	.29±.00	41.2±2.0
2	1.5	8.73±.06	.97±.26	36.8±.7
	3.0	9.00±.46	.16±.05	76.6±4.4
	4.5	8.99±.14	.17±.04	89.2±15.7
3	1.5	8.81±.04	.44±.04	53.8±1.2
	3.0	8.86±.14	.30±.07	62.5±3.9
	4.5	9.22±.12	.26±.08	28.7±7.0

Chemical Analysis of Lysimeter Extracts

As mentioned earlier, the lysimeters used in this study were designed especially for the collection of microbiological data. As such, they could not be operated under conditions of continuous tension. Further, as relatively large samples were required for microbiological analyses, sampling could occur only when irrigation rates were high enough to saturate the soil in the vicinity of a particular lysimeter. These restrictions limited the consistency of sample collection and the amount of data available for analysis among lysimeters. For example, samples were collected from lysimeter site #3 on only four occasions while samples were available for analysis from lysimeter site #1 on as many as 15 occasions. Data collected from the soil moisture probes throughout this study support this contention. Specifically, the soil adjacent to the lysimeters at site #1 was saturated more frequently than were those at sites 2 and 3. Further, the deeper lysimeters were saturated more frequently than were the shallower ones.

Typical values for the analysis of lysimeter samples can be found in Table 40. In general, these mean values indicate the chemical quality of the lysimeter samples to be somewhat improved over the wastewater in the irrigation pond. There are exceptions to this statement. For example, the nitrate-nitrogen concentrations of the water from the lysimeters was found to be consistently higher than that of the irrigation pond water. This coupled with a decrease in total Kjeldahl nitrogen should not be unexpected as such results have been observed at many other sites. The higher solids levels found (particularly at the lysimeter site #1) are not reflective of the wastewater applied but rather of the soil conditions at the site, the lysimeter construction and the sampling procedures.

Conclusions regarding wastewater renovation as a function of depth cannot be drawn from these data. To evaluate these processes, it was necessary to use data from the special irrigation studies only. Experimental procedures can be found in the METHODS AND MATERIALS section.

Tables 41 and 42 present the chemical and physical data developed during both special irrigation studies. These data support the theory of renovation of the wastewater applied to these soils with regard to total phosphate, orthophosphate, ammonia-nitrogen and in most cases, total Kjeldahl nitrogen. Under some conditions, depth appeared to play a role. Little can be said regarding the effect of the soils or depth on total organic carbon concentration. In the case of nitrate-nitrogen, major increases were observed in the waters from the lysimeters. These increased concentrations can be a direct result of the conversion (oxidation) of other nitrogen forms as well as leaching of the soil. The latter explanation is supported by the accompanying conductivity data.

Microbiological Analyses

The lysimeter microbiological data are of greater potential interest than are the soil studies because of the possible opportunity they

TABLE 40. Mean Results of Chemical/Physical Analyses, Lysimeter--Kerrville Site

Parameter (mg/l)	Irrigation Pond	Lysimeter Site #1			Lysimeter Site #2			Lysimeter Site #3		
		1.5 ft.	3.0 ft.	4.5 ft.	1.5 ft.	3.0 ft.	4.5 ft.	1.5 ft.	3.0 ft.	4.5 ft.
Total Organic Carbon	21	18	18	17	28	14	13	10	13	13
Orthophosphate (as P)	5.4	5.6	5.4	4.0	1.8	2.5	1.7	2.4	<0.22	0.55
Total Phosphate (as P)		7.4	7.0	5.3	2.4	2.7	1.9	0.8	1.5	0.8
Total Kjeldahl Nitrogen (as N)	6.2	3.6	4.0	4.2	0.9	1.0	1.0	1.1	1.1	1.8
Nitrate-Nitrogen	4.9	18.1	15.0	10.7	6.9	10.7	13.8	1.1	11.6	18.0
Ammonia-Nitrogen	2.9	2.1	2.0	2.6	0.21	<0.06	0.21	<0.10	0.40	0.24
Nitrite-Nitrogen	2.0	1.6	2.5	1.2	0.65	<0.06	<0.06	<0.10	0.04	0.02
Total Suspended Solids	39	64	126	96	21	7.5	7.5	30	18	16
Total Volatile Suspended Solids	30	27	35	22	11	3.4	4.1	16	18	16
% Volatile	(77)	(42)	(28)	(23)	(52)	(45)	(55)	(45)	(100)	(100)
pH (units)	8.2	7.7	7.6	7.4	7.7	7.4	7.5	-	-	-
Conductivity (μmhos/cm)		966	1043	1019	1108	970	976	-	-	-

TABLE 41. Chemical/Physical Data, Special Irrigation Study I, Kerrville Site

Parameter	1-31-78				2-2-78			
	Irrigation Pond	Lysimeter #1 (depth)			Irrigation Pond	Lysimeter #1 (depth)		
		1.5 ft.	3.0 ft.	4.5 ft.		1.5 ft.	3.0 ft.	4.5 ft.
Total Organic Carbon (mg/l)	22	12	12	12	18	10	14	14
Total Phosphate (mg/l)	14	9.5	9.0	9.5	9.0	8.5	8.0	8.5
Orthophosphate (mg/l)	12	7.5	7.5	7.5	8.0	7.5	7.5	7.5
Total Kjeldahl Nitrogen (mg/l)	16	8.0	8.0	8.0	16	8.0	8.0	13
Ammonia-N (mg/l)	14	6.6	5.0	6.4	15	8.0	6.0	8.0
Nitrate-N (mg/l)	9.5	15.7	14.7	15.5	7.4	12.2	13.2	12.5
Nitrite-N (mg/l)	1.5	1.3	1.3	1.5	0.63	0.75	0.75	0.50
pH	7.9	7.4	7.4	7.5	7.9	7.7	7.5	7.7
Conductivity (μmhos/cm)	1,900	2,000	2,000	2,000	1,680	1,680	1,680	1,700

TABLE 42. Chemical/Physical Data, Special Irrigation Study II--Kerrville Site

Parameter	5-1-78				5-3-78				5-5-78			
	Irrigation Pond	Lysimeter #2 (depth)			Irrigation Pond	Lysimeter #2 (depth)			Irrigation Pond	(Lysimeter #2 (depth)		
		1.5 ft.	3.0 ft.	4.5 ft.		1.5 ft.	3.0 ft.	4.5 ft.		1.5 ft.	3.0 ft.	4.5 ft
Total Organic Carbon (mg/L)	18	34	27	14	25	30	24	22	23	30	27	20
Total Phosphate (mg/L)	10.4	2.2	3.4	2.6	10.0	4.5	4.2	4.0	10.0	4.0	4.4	4.2
Orthophosphate (mg/L)	9.4	1.4	2.6	0.7	9.2	3.8	3.5	1.5	9.2	2.2	2.9	1.7
Total Kjeldahl Nitrogen (mg/L)	3.5	6.2	6.2	2.4	7.5	6.0	4.0	2.4	8.0	5.4	3.9	2.2
Ammonia-N (mg/L)	0.2	0.35	0.16	0.02	1.1	0.13	0.02	0.01	1.8	0.05	0.02	0.01
Nitrate-N (mg/L)	16.0	37.6	19.6	23.0	14.0	28.9	16.0	18.0	13.0	21.3	17.9	16.0
Nitrite-N (mg/L)	0.03	0.38	0.43	0.03	0.05	0.09	0.02	0.02	0.03	0.69	0.11	0.02
pH	7.6	7.6	7.5	7.4	8.0	7.2	7.0	7.0	7.7	7.4	7.4	7.2
Conductivity (µmhos/cm)	1495	2150	1650	1600	1450	2075	1650	1580	1445	2000	1575	1575

present for assessing the degree of movement of indicator bacteria, coliphages, and enteric viruses from the soil surface. To this end, the three lysimeter locations were chosen to represent distinctly different soil types at the Kerrville irrigation site. Unfortunately, samples available from lysimeter #3 were sporadic and, therefore, of limited interpretative value. Only lysimeters located at sites #1 and #2 were amenable to regular sampling.

Prior to assessing field data, a laboratory experiment was conducted to evaluate the potential for regrowth of indicator organisms in lysimeter waters. Samples were collected aseptically from the 1.5 and 3.0 feet depths at lysimeter #1. Immediately upon receipt in the laboratory, indigenous levels of total coliforms and fecal streptococci were determined by appropriate membrane filtration techniques. Aliquots of the original samples were held in the dark at 4C, 20C, and 35C, and subsequently assayed at 2, 3, 4, 5, 6, 7 and 10 days. The data accrued from this simulated testing are compiled in Appendix B. Representative results, graphed as fecal streptococcal inactivation are shown in Figure 16. Organism die-away (as measured by recoverability) was observed at all temperatures over the 10-day monitoring period. From these tests, it was concluded that insufficient nutrients coupled with existing environmental conditions were such that regrowth in lysimeters was not a significant factor to consider in evaluating field data.

An overview of the lysimeter field data is presented in Table 43 as ranges of results for the bacterial and viral analyses completed during an eighteen-month period. Generally, bacteria and coliphage densities were highest in the water collected at lysimeter #1. Although enteric virus isolations were made on at least one occasion at each site, the most frequent recoveries occurred at lysimeter #2 (six water samples yielding virus) and lysimeter #1 (3 water samples yielding virus). These positive recoveries involved the concentration from volumes ranging from 1 liter to 20 liters. Interestingly, viruses were detected in water sampled to a depth of 3 feet at lysimeter #1 and 4.5 feet at lysimeter #2. These results contrast with the poor rate of recovery of viruses from soils in the lysimeter area and are remarkable in view of the low levels of viruses reported for the irrigation pond (Table 22).

Specific organism levels at each lysimeter for selected sampling dates are shown in Table 44. The July, 1977 data were accumulated under typical conditions of irrigation while the August, 1978 sampling was done immediately after heavy rainfall and accompanying flooding. In almost all cases, when samples were obtained at all three depths, little attenuation of organism density was evident through 4.5 feet of soil.

An exception can be seen in the temporal sampling beginning on August 2, 1978 at lysimeter #2. In the absence of site irrigation during flooding, bacterial and coliphage counts were highest on the first day of sampling with viruses being recovered at all three depths. Over the following two days, organism concentrations decreased with both depth and time. During the same period, bacteria and coliphage levels in samples obtained at lysimeter #1 decreased with time, but no attenuation through soil was observed at lysimeter #1 decreased with time, but no

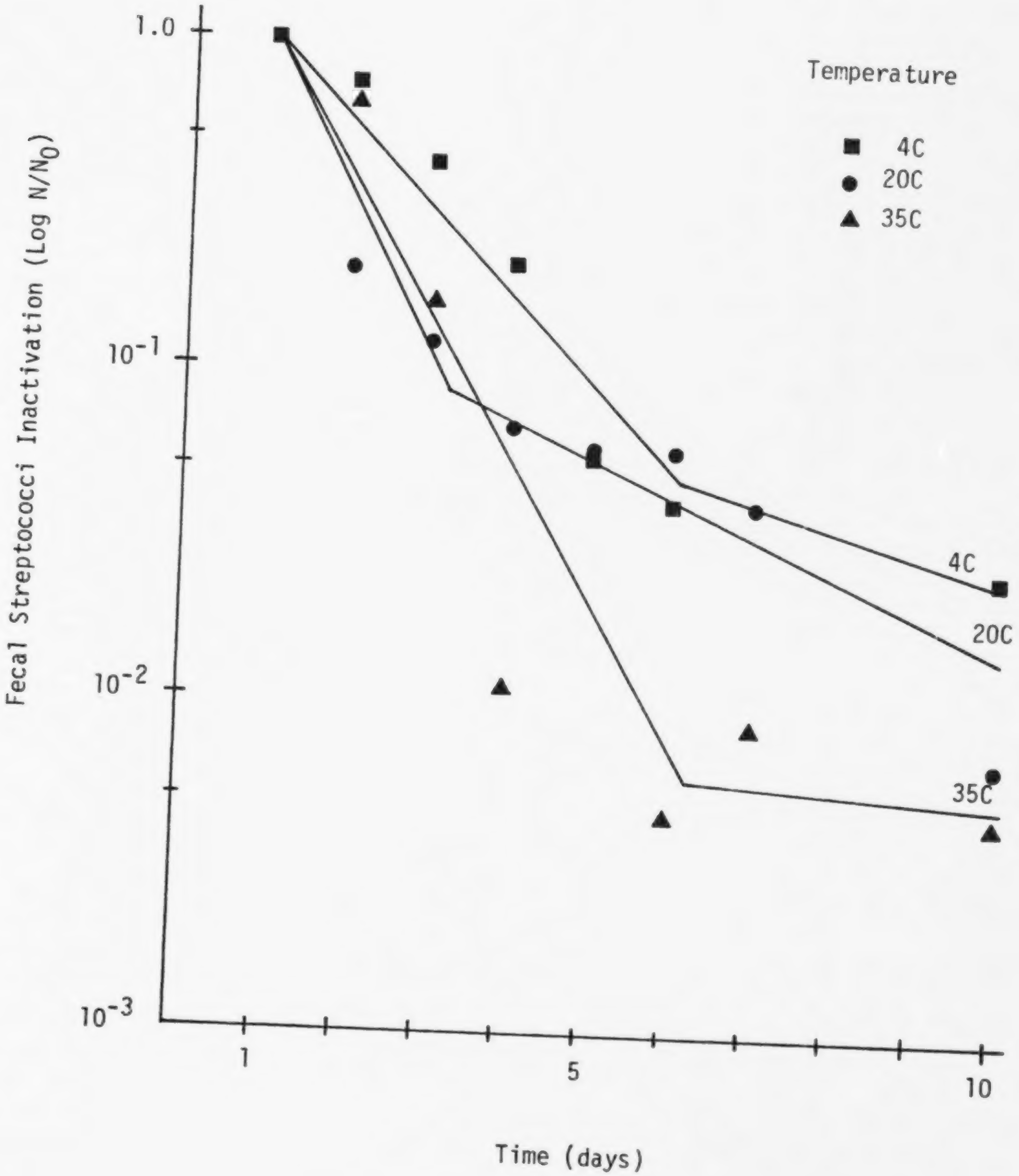

FIGURE 16. Fecal Streptococci Inactivation in Lysimeter Water

TABLE 43. Ranges of Results of Bacteria and Bacteriophage Analyses of Lysimeter Samples, Kerrville Site*

Location	Total Coliforms (cfu/100 ml)	Fecal Coliforms (cfu/100 ml)	Fecal Streptococci[a] (cfu/100 ml)	Bacteriophage (pfu/liter)	Remarks
Lysimeter #1					
1.5 ft.	$1.8x10^{4} - 3.9x10^{6}$	$6.3x10^{3} - 8.1x10^{5}$	$1.7x10^{3} - 2.0x10^{5}$	$5.0x10^{1} - 2.2x10^{5}$	1 sample positive for virus (1-NI)
3.0 ft.	$2.0x10^{3} - 5.6x10^{6}$	$3.3x10^{2} - 1.0x10^{6}$	$2.7x10^{2} - 5.4x10^{5}$	$5.0x10^{1} - 4.9x10^{5}$	2 samples positive for virus (1-CB5, 1 E-11, 1 E-21)
4.5 ft.	$1.1x10^{3} - 3.1x10^{6}$	$1.0x10^{2} - 7.8x10^{5}$	$6.7x10^{1} - 9.2x10^{5}$	$<5.0x10^{1} - 5.9x10^{5}$	No positive viral isolates
Lysimeter #2					
1.5 ft.	$1.7x10^{2} - 4.3x10^{5}$	$3.0x10^{1} - 3.2x10^{4}$	$<3.3x10^{-1} - 1.1x10^{4}$	$<5.0x10^{1} - 4.9x10^{4}$	1 sample positive for virus (1-CB4, 2-CB5)
3.0 ft.	$6.0x10^{3} - 2.2x10^{6}$	$5.5x10^{2} - 1.5x10^{5}$	$1.0x10^{2} - 1.2x10^{4}$	$5.0x10^{1} - 4.4x10^{5}$	2 samples positive for virus (1-polio 1, 1-CB4)
4.5 ft.	$3.3x10^{1} - 4.5x10^{5}$	$<3.3x10^{0} - 4.0x10^{4}$	$<3.3x10^{0} - 2.1x10^{4}$	$<1.0x10^{2} - 9.8x10^{3}$	3 samples positive for virus (16-polio 1, 1-CB3, 1-CB4, 1-NI)
Lysimeter #3					
1.5 ft.	$2.3x10^{2} - 2.7x10^{6}$	$1.3x10^{2} -> 3x10^{5}$	Insufficient data	$1.0x10^{2} - 8.0x10^{3}$	1 sample positive for virus (1-polio 1)
3.0 ft.	$2.6x10^{1} - 6.7x10^{5}$	$6.0x10^{1} - 3.3x10^{4}$		$2.5x10^{2} - 3.1x10^{5}$	No positive viral isolates
4.5 ft.	$<3.3x10^{0} - 1.2x10^{6}$	$<3.3x10^{0} - 1.3x10^{5}$		$<1.0x10^{2} - 1.5x10^{3}$	No positive viral isolates

* Reporting period 2/77 - 8/78

[a] Testing for this indicator initiated 7/77

NI-virus confirmed by tube culture passage, but not identified.

TABLE 44. Selected Results of Lysimeter Microbiological Analysis

Parameter	Depth (ft)	Lysimeter #1					Lysimeter #2				Lysimeter #3		
		7/12/77	7/14/77	8/2/78[β]	8/3/78	8/4/78	7/28/77	8/2/78[β]	8/3/78	8/4/78	7/12/77	7/14/77	8/2-4/78[β]
Total Coliforms (cfu/100 ml)	1.5	5.2×10^{5}	5.6×10^{5}	3.9×10^{6}	8.0×10^{5}	2.8×10^{5}	3.7×10^{5}	2.9×10^{5}	2.2×10^{5}	1.1×10^{5}	3.6×10^{5}	8.5×10^{5}	
	3.0	6.1×10^{5}	4.4×10^{5}	5.6×10^{6}	4.6×10^{5}	9.2×10^{5}	2.2×10^{6}	1.8×10^{5}	4.8×10^{4}	6.0×10^{3}	4.8×10^{5}	3.3×10^{5}	
	4.5	5.9×10^{4}	3.3×10^{4}	3.1×10^{6}	3.2×10^{6}	3.4×10^{5}	1.7×10^{5}	9.6×10^{4}	4.0×10^{4}	4.1×10^{3}	4.1×10^{5}	1.3×10^{5}	
Fecal Coliforms (cfu/100)	1.5	2.7×10^{4}	*	8.1×10^{5}	2.2×10^{5}	6.2×10^{4}	3.2×10^{4}	2.9×10^{4}	2.7×10^{4}	1.0×10^{4}	2.4×10^{4}	*	
	3.0	1.7×10^{5}	*	1.0×10^{6}	3.0×10^{5}	1.5×10^{5}	1.5×10^{5}	1.6×10^{4}	8.3×10^{3}	1.1×10^{3}	1.9×10^{4}	*	
	4.5	1.7×10^{4}	*	7.8×10^{5}	3.5×10^{5}	1.2×10^{5}	3.3×10^{3}	1.3×10^{4}	9.0×10^{3}	6.0×10^{2}	1.7×10^{4}	*	
Fecal Streptococci (cfu/100 ml)	1.5	Not Done	Not Done	2.0×10^{5}	9.4×10^{4}	1.3×10^{4}	1.1×10^{4}	6.3×10^{3}	1.0×10^{4}	1.7×10^{3}	Not Done	Not Done	
	3.0			5.4×10^{5}	3.3×10^{5}	5.7×10^{4}	1.2×10^{4}	1.6×10^{3}	2.0×10^{3}	1.0×10^{2}			
	4.5			4.8×10^{5}	9.2×10^{5}	8.4×10^{4}	1.1×10^{3}	1.6×10^{3}	4.9×10^{2}	2.3×10^{1}			
Bacteriophage (pfu/liter)	1.5	4.0×10^{3}	4.5×10^{3}	2.2×10^{5}	1.8×10^{4}	8.0×10^{3}	1.0×10^{4}	4.9×10^{4}	9.2×10^{3}	6.6×10^{3}	3.5×10^{2}	4.0×10^{2}	
	3.0	7.9×10^{3}	3.7×10^{3}	4.9×10^{5}	5.4×10^{4}	1.7×10^{4}	9.5×10^{2}	3.3×10^{4}	1.0×10^{3}	$<2.0\times10^{2}$	6.5×10^{2}	µ	
	4.5	2.0×10^{2}	3.0×10^{2}	5.9×10^{5}	1.4×10^{5}	1.2×10^{4}	3.5×10^{2}	2.5×10^{4}	4.6×10^{3}	$<2.0\times10^{2}$	4.5×10^{2}	$<1.0\times10^{2}$	
Enteric Viruses (confirmed isolates+)	1.5	-	-	+ (1-NI)[α]	-	-	µ	+ (3-CB5)	-	-	-	µ	
	3.0	-	-	-	-	-	µ	+ (1-CB4)	-	-	-	µ	
	4.5	-	-	-	-	-	µ	+ (1-CB3, 1-CB4)	-	-	-	µ	

* Assay lost due to water leakage into dishes.

NI-Virus confirmed by tube culture passage, but not identified.

µ No sample.

+ All confirmed viruses recovered by concentration of 20 liter volumes.

β Heavy rainfall beginning 8/1/78, >10 inches recorded on site within 12 hours. Extensive flooding of rivers; Third Creek broke through levee at two points making Lysimeter #3 inaccessible.

attenuation through soil was observed. Once again, a positive viral sample was obtained on the first day of sampling following the heavy rainfall.

In an effort to assess more closely the effects of effluent irrigation at the Kerrville site, a series of coordinated studies involving intensive sampling of lysimeters during scheduled irrigation were conducted. The first study was done in an area around lysimeter #1 with water samples from lysimeters and the irrigation pond being collected after approximately six hours of irrigation. The irrigation schedule shown in Table 45 reflects a total of 24.6 inches of secondary effluent applied in the field during the study. Data presented in Table 46 profiles the microbiological results from this first study. If one considers the levels of organisms detected in irrigated effluent and water collected from lysimeters, significant microbial penetration in the saturated soil zone to 4.5 feet was observed. Using the level of these indicator organisms in effluent grab samples from the irrigation pond as a general measure of application, the bacterial removals observed ranged from no reduction to 50% removal. Similarly, a comparison of coliphage levels in irrigation pond effluent and lysimeter water samples show a maximal reduction of 35% in water moving through the soil profile. Finally, although no enteric viruses were recovered from irrigation pond grab samples, two confirmed viral isolates were detected at a lysimeter depth of three feet in the January 31 sample. The viruses picked from HeLa cell monolayers were identified as ECHO 11 and ECHO 21.

A second intensive irrigation study was carried out at the Kerrville site in the general area of lysimeter #2. Because of the interesting findings of the first study, more emphasis was placed on lysimeter sampling. Over the ten-day study period, a minimum of 18 inches of secondary effluent and 1.9 inches of rainfall were measured in the study area. Microbiological results are outlined in Table 47. Bacterial retention within the soil profile in the second study area appeared to be better than in the area of lysimeter #1, perhaps due in part to the decreased hydraulic loading in the second study. Levels of fecal coliform and fecal streptococci generally were reduced by more than 90% in lysimeter waters within the first 1.5 feet. However, attenuation within the soil profile was sporadic. Interestingly, the levels of organisms at all depths decreased with each sampling time even though levels in the irrigation pond were relatively consistent

As in the previous special irrigation study, both bacteriophage and enteric viruses were detected in water intercepted below 1.5 feet. Enteric virus isolations (as plaque-forming units) were confirmed off both HeLa and BGM cell lines. On the first day of lysimeter sampling 32 pfu of poliovirus 1 were recovered from the irrigation pond in a 20-liter grab sample. Additionally, one viral isolate was confirmed from the 3.0-foot depth and 11 pfu from 4.5 feet. No enteric virus isolations were obtained in subsequent testing. Further discussion of these isolates can be found under VIRAL RECOVERIES, IDENTIFICATION AND CHARACTERIZATION.

Overall, microbial analyses of water collected from lysimeters indicate a movement of both indicator organisms and enteric viruses

TABLE 45. Irrigation Schedule for Special Irrigation Study I, Kerrville Site

	Sunday 1/29/78	Monday 1/30/78	Tuesday 1/31/78	Wednesday 2/1/78	Thursday 2/2/78	Friday 2/3/78
Rainfall (inches)	0.3	0	0.2	0	0	0
Irrigation (inches)	7.2	0	10.8	0	6.6	0
Total Applied (inches)	7.5	0	11.0	0	6.6	0

TABLE 46. Microbiological Data, Special Irrigation Study I, Kerrville Site

Parameter	1-31-78				2-2-78			
	Irrigation Pond	Lysimeter #1 (depth)			Irrigation Pond	Lysimeter #1 (depth)		
		1.5 ft.	3.0 ft.	4.5 ft.		1.5 ft.	3.0 ft.	4.5 ft.
Total Coliform (cfu/100 ml)	1.2×10^6	1.0×10^6	8.4×10^5	9.0×10^5	1.4×10^6	7.3×10^5	7.9×10^5	7.2×10^5
Fecal Coliform (cfu/100 ml)	4.4×10^5	4.2×10^5	4.0×10^5	3.8×10^5	4.3×10^5	3.0×10^5	2.9×10^5	3.9×10^5
Fecal Streptococci (cfu/100 ml)	5.2×10^3	7.7×10^3	8.3×10^3	6.4×10^3	5.5×10^3	4.0×10^3	9.2×10^3	2.7×10^3
Bacteriophage (pfu/liter)	2.0×10^4	1.8×10^4	1.6×10^4	1.4×10^4	2.0×10^4	1.4×10^4	1.3×10^4	1.7×10^4
Enteric Viruses (pfu/liter)	<.11	<.11	0.21*	<.11	<.11	<.11	<.11	<.11

*Two viral isolates identified as: 1-ECHO 11, 1-ECHO 21

TABLE 47. Microbiological Data, Special Irrigation Study II--Kerrville Site

Parameter	5-1-78				5-3-78				5-5-78			
	Irrigation Pond	Lysimeter #2 (depth)			Irrigation Pond	Lysimeter #2 (depth)			Irrigation Pond	Lysimeter #2 (depth)		
		1.5 ft.	3.0 ft.	4.5 ft.		1.5 ft.	3.0 ft.	4.5 ft.		1.5 ft.	3.0 ft.	4.5 ft.
Total Coliform (cfu/100 ml)	$7.9x10^5$	$4.3x10^5$	$7.5x10^5$	$2.4x10^5$	$5.1x10^5$	$2.6x10^4$	$3.8x10^4$	$5.3x10^4$	$1.8x10^5$	$7.3x10^3$	$6.5x10^4$	$2.4x10^3$
Fecal Coliform (cfu/100 ml)	$1.1x10^5$	$1.4x10^4$	$1.2x10^4$	$4.0x10^3$	$4.8x10^4$	$3.9x10^2$	$5.5x10^2$	$8.9x10^2$	$5.6x10^4$	$2.4x10^2$	$4.1x10^3$	$1.0x10^2$
Fecal Streptococci (cfu/100 ml)	$9.9x10^3$	$5.3x10^3$	$5.6x10^3$	$2.9x10^3$	$3.4x10^3$	$3.5x10^2$	$4.3x10^2$	$2.4x10^2$	$5.0x10^3$	$4.3x10^1$	$3.2x10^3$	$1.0x10^1$
Bacteriophage (pfu/liter)	$7.3x10^4$	$1.6x10^4$	$4.4x10^4$	$7.8x10^3$	$2.3x10^4$	$1.6x10^3$	$<2.0x10^2$	$4.0x10^2$	$6.9x10^4$	$<2.0x10^2$	$2.4x10^3$	$2.0x10^2$
Enteric Viruses$^{\alpha}$ (pfu/liter)	$2.1x10^0$	β	$1.0x10^{-1}$	$8.5x10^{-1}$	$<1.3x10^{-1}$	β	$<1.0x10^{-1}$	$<1.0x10^{-1}$	$<1.1x10^{-1}$	β	$<1.2x10^{-1}$	$<1.2x10^{-1}$

α results from Hela and BGM plaque assay system; all values represent confirmed viral isolates from both cell lines.

β insufficient sample volume.

to a sampling depth of at least 4.5 feet under an effluent irrigated surface when relatively intensive irrigation schedules are maintained. Unfortunately, these observations are not readily explained. Possibilities include the specific nature of the subsurface soils, cracking of the surface soils, or virus adsorption/elution as a result of high hydraulic loading (both precipitation and irrigation).

MONITORING WELLS

Well Cuttings Study

Samples were collected from all five monitoring wells at 2-foot intervals during drilling operations. The wells were drilled with a rotary rig and a natural drilling mud. Samples were collected from the drilling cuttings as they returned to the surface. The cuttings were analyzed and a lithologic log prepared for each well. Binocular microscope studies were made of the cuttings to determine lithology, texture, and mineralogocal content. The alluvial material in the well cuttings was generally shattered and broken by the rotary drilling operations and no accurate dexcription of size, sorting or degree of cementation could be made.

In addition to the cuttings analysis, an offset study of the alluvial gravel in wells #2, 3, and 4 was performed using an exposed outcrop of alluvial materials in the stream bed of Third Creek immediately to the west of well #3. The alluvial gravels are widespread in the vally and the outcrop is considered to be representative of the alluvial gravels in the wells. The results of this study are included in the lothologic logs, Table 48.

Hydrogeology

Groundwater occurs under water table conditions throughout the portion of the valley of Third Creek which contains alluvial deposits of gravel, sand, silt and clay. Wastewater which is applied as irrigation enters this shallow groundwater system and is confined to the alluvial sediments. The marls of the underlying Upper Glen Rose Formation act as an aquiclude and effectively prevent downward migration of groundwater from the alluvial sediments in the valley fill to the deeper limestone formations. No structural faulting or jointing was evident in exposures of the Upper Glen Rose Formation in the study area.

The alluvial materials are highly variable both horizontally and vertically with interfingering, discontinuous leases of clay, silt, sand and gravel. The thickness of the sand and gravel layers may vary from a few centimeters to 40 or 50 centimeters. Because of the extreme variability of the alluvial sediments, these materials are anisotropic to groundwater flow, resulting in a wide range of yields for wells completed within the same interval.

Typical water level contours at the Kerrville site as shown in Figure 17 indicate that the groundwater in the valley alluvium moves in a west to southwesternly direction towards Third Creek. The water level contours are based on water level measurements in the observation wells.

TABLE 48. Lithologic Logs of Monitoring Wells

Thickness (ft)	Depth (ft)	Lithology
		Well #1
1	1	Surface soil, light yellow to brown loam. Calcareous. Small limestone fragments (10%) 2-7 mm diameter.
2	3	Loam, light yellow. Numerous limestone fragments (60%) 2-10 mm diameter. Calcareous.
5	9	Limestone, thin bedded, weakly cemented Biomicrite.
29	38	Marl (a soft, loose, earthy deposit consisting chiefly of 35-65% carbonate and 36-65% clays). Yellow, plastic. Some limestone fragments, especially in upper portions.
27	65	Marl, gray, plastic, low permeability.
Total Depth = 65 feet		
		Well #2
1	1	Surface soil. Dark gray, silty clay. Few $CaCO_3$ concretions. Calcareous.
15	16	Gray to brown silty clay. Few limestone fragments (<10%). Calcareous. Very plastic.
12	28	Gravel, limestone (60%) and chert (40%) 2-20 mm diameter, rounded, moderate to poor sorting. Some calcite cementation rinds. High porosity.
5	45	Marl, gray, very plastic. Low porosity.
Total Depth = 45 feet		
		Well #3
8	8	Surface soil. Pale gray to very dark brown silty clay and sandy loam. Calcareous. Few (2-3%) small rounded limestone pebbles.
26	34	Gravel, limestone (60%) and chert (40%), 2-20 mm diameter. Rounded, moderate to poor sorting. Some calcite cementation rinds. High porosity.
1	35	Marl, gray, very plastic. Low porosity.
Total Depth = 35 feet		

TABLE 48. Lithologic Logs of Monitoring Wells (Continued)

Thickness (ft)	Depth (ft)	Lithology
		Well #4
10	10	Surface soil. Light brown to gray. Sandy loam. Calcareous. Few (5%) rounded limestone pebbles 2-10 mm diameter.
6	16	Light brown silty clay. Gravelly, numerous limestone and calcite pebbles. Pebbles rounded 2-10 mm diameter.
24	40	Gravel, limestone (60%) and chert (40%) 2-20 mm diameter. Rounded moderate to poor sorting. Some calcite cementation rinds. High porosity.
5	45	Marl, gray, very plastic. Low porosity.
Total Depth = 45 feet		
		Well #5
1	1	Surface soil light brownish gray loam. 2-5% small limestone fragments.
19	19	Limestone, thinly bedded. Weakly cemented, interbedded with marl and clay. Biomicrite. Moderate to low porosity.
6	25	Marl, yellow, very plastic. Low porosity.
20	45	Marl, gray, very plastic. Low porosity.
Total Depth = 45 feet		

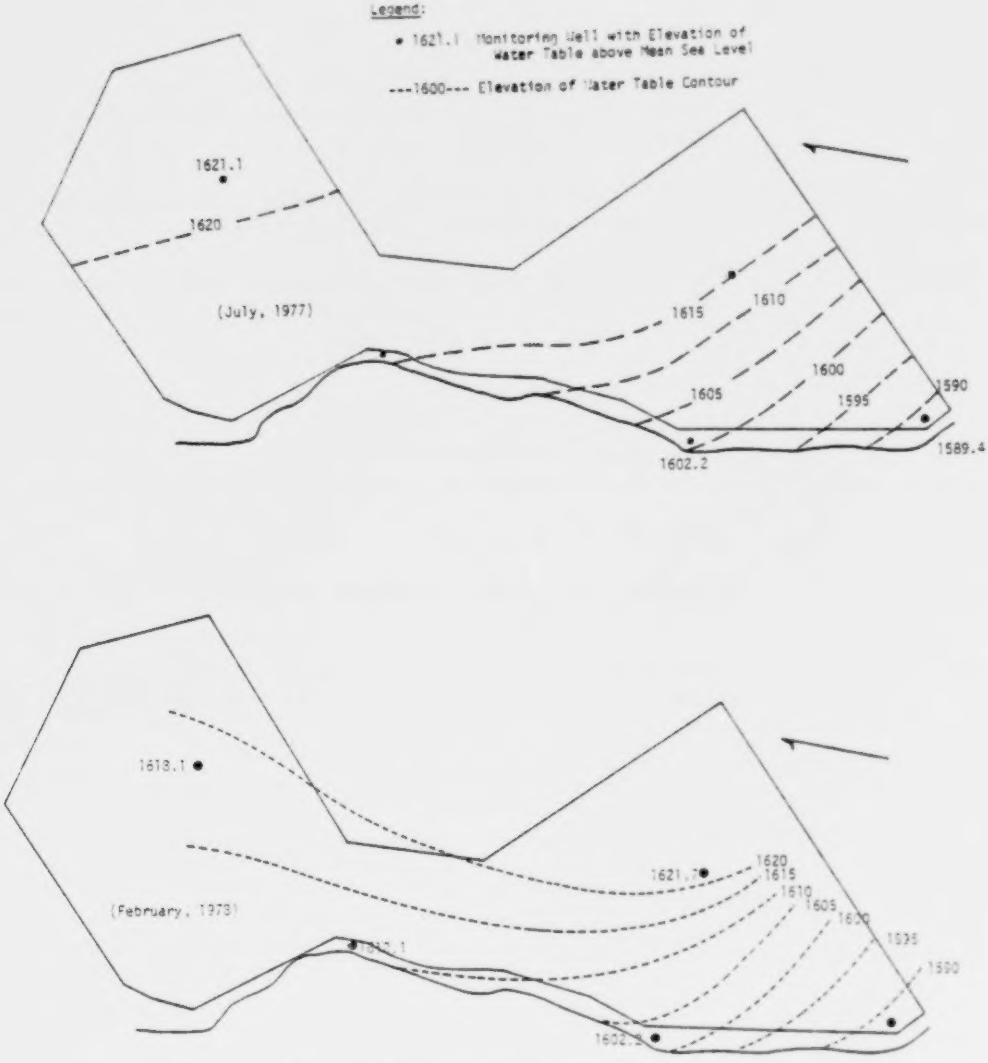
Legend:
• 1621.1 Monitoring Well with Elevation of Water Table above Mean Sea Level
---1600--- Elevation of Water Table Contour
1621.1
1620
(July, 1977)
1615
1610
1605
1600
1595
1590
1602.2
1589.4
1618.1
1621.7
(February, 1978)
1620
1615
1610
1605
1600
1595
1590

FIGURE 17. Groundwater Contours, Kerrville Site

Movement of the groundwater is generally toward Third Creek where it is either discharged to the creek as seeps and springs or continues as subsurface flow in the alluvium down-valley. Stream flow measurements at the upstream and downstream limits of the site indicate that there is an average increase in stream flow of 245 gpm (0.35 mgd) due to groundwater being discharged to the stream. Additional groundwater is discharged downstream from the site as indicated by the numerous springs and seeps in the valley area below the downstream gauging station.

The water levels in the observation wells varied greatly in their response to the application of irrigation water and rainfall. Figure 18 summarizes the precipitation and irrigation at the Kerrville site. Wells #1 and 5, which were completed primarily in the Upper Glen Rose Formation and upslope from the major irrigation areas displayed the smallest change in water levels (see Figures 19 and 20). Wells #3 and 4 are completed entirely in the alluvial materials and display the greatest changes in water levels in response to irrigation or rainfall as is shown in Figure 19.

Comparison of the water levels in each well with the irrigation and precipitation recorded during the study (Figure 18) indicate that the response of Wells #3 and 4 is closely related to irrigation and/or precipitation episodes. The water level contours indicate that a large amount of the groundwater which originates as either irrigation or precipitation on the site will move in the alluvium past wells #3 and 4. On the other hand, the response of Wells #1 and 2 is not correlated with irrigation/precipitation episodes, thereby indicating that groundwater flow to those wells is not directly related to precipitation or irrigation in the immediate vicinity of the wells.

Chemical and Physical Analysis

The hydraulic response of the monitoring wells completed in the valley alluvium demonstrates that the groundwater system responds to the application of wastewater as irrigation and to precipitation on the site. The groundwater was sampled at intervals during the study to determine the quality of the groundwater and to compare groundwater quality with the wastewater applied as irrigation. Results of the chemical and physical analyses of the groundwater can be found in Tables 49 and 50. These data indicate that significant renovation of the wastewater has occurred after vertical infiltration through approximately 250 cm of soil and horizontal movement through the sand, silts and gravels of the alluvial material.

That the wastewater renovation from a chemical viewpoint has been relatively effective is clearly indicated by the levels of TOC, Kjeldahl nitrogen, and ortho- and total phosphate found in the well waters. The fact that higher levels of nitrate-nitrogen were found in wells #1 and 5 may be explained by the lack of a crop in these areas, thereby precluding significant nitrogen uptake. It also should be noted that the ammonia-nitrogen level was highest in well #1.

The solids and turbidity data of Table 49 are reflective of both

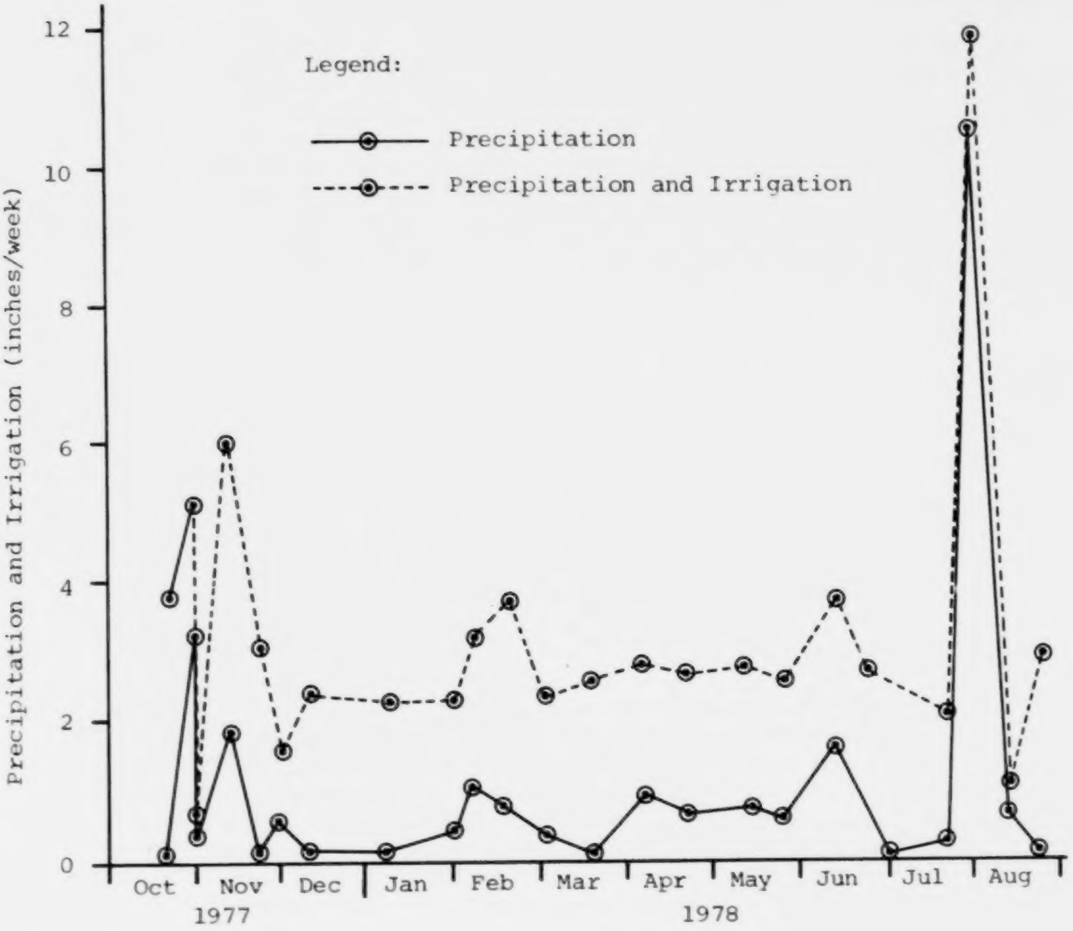

FIGURE 18. Summary of Precipitation and Irrigation, Kerrville Site

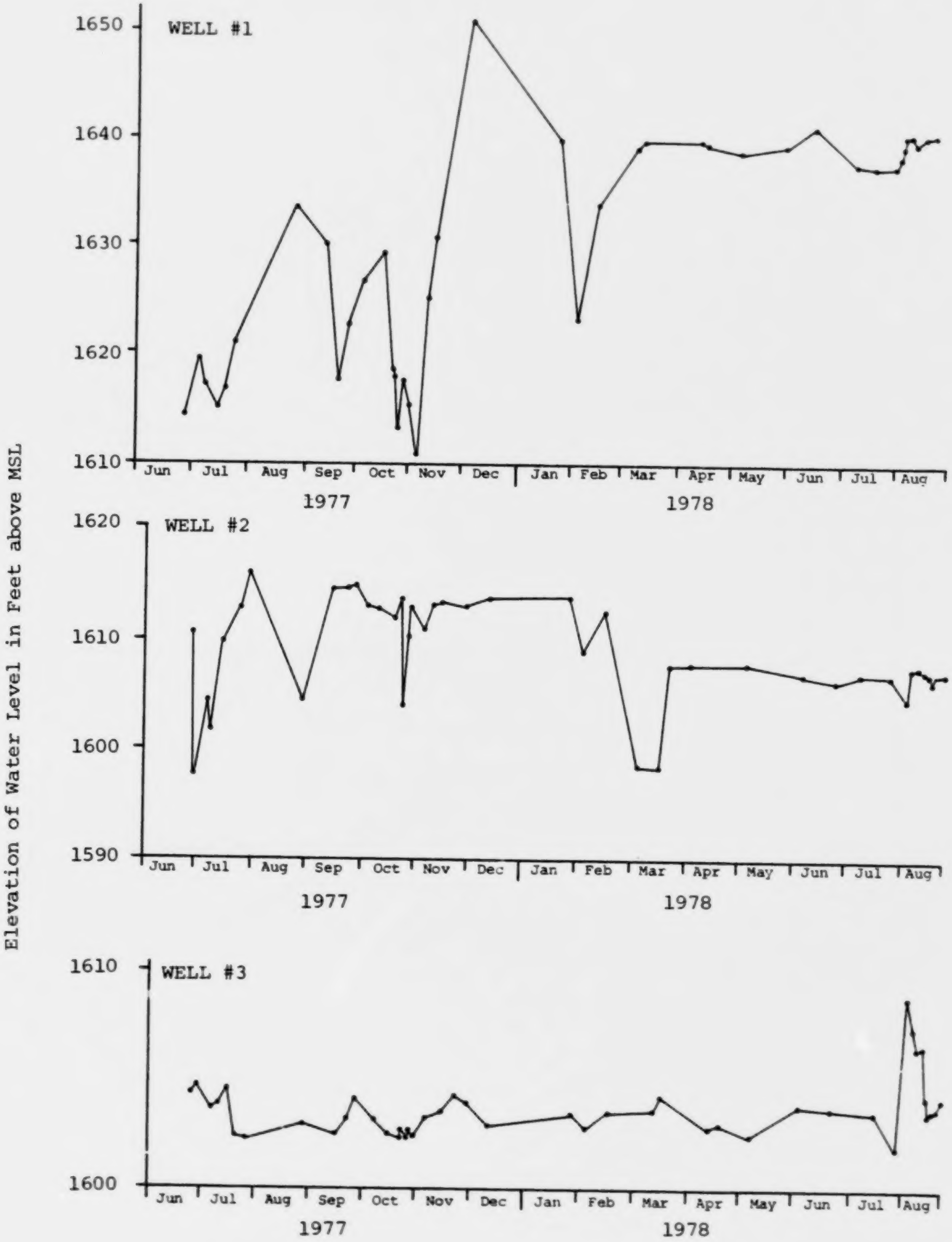

FIGURE 19. Hydrographs of Wells 1, 2 and 3

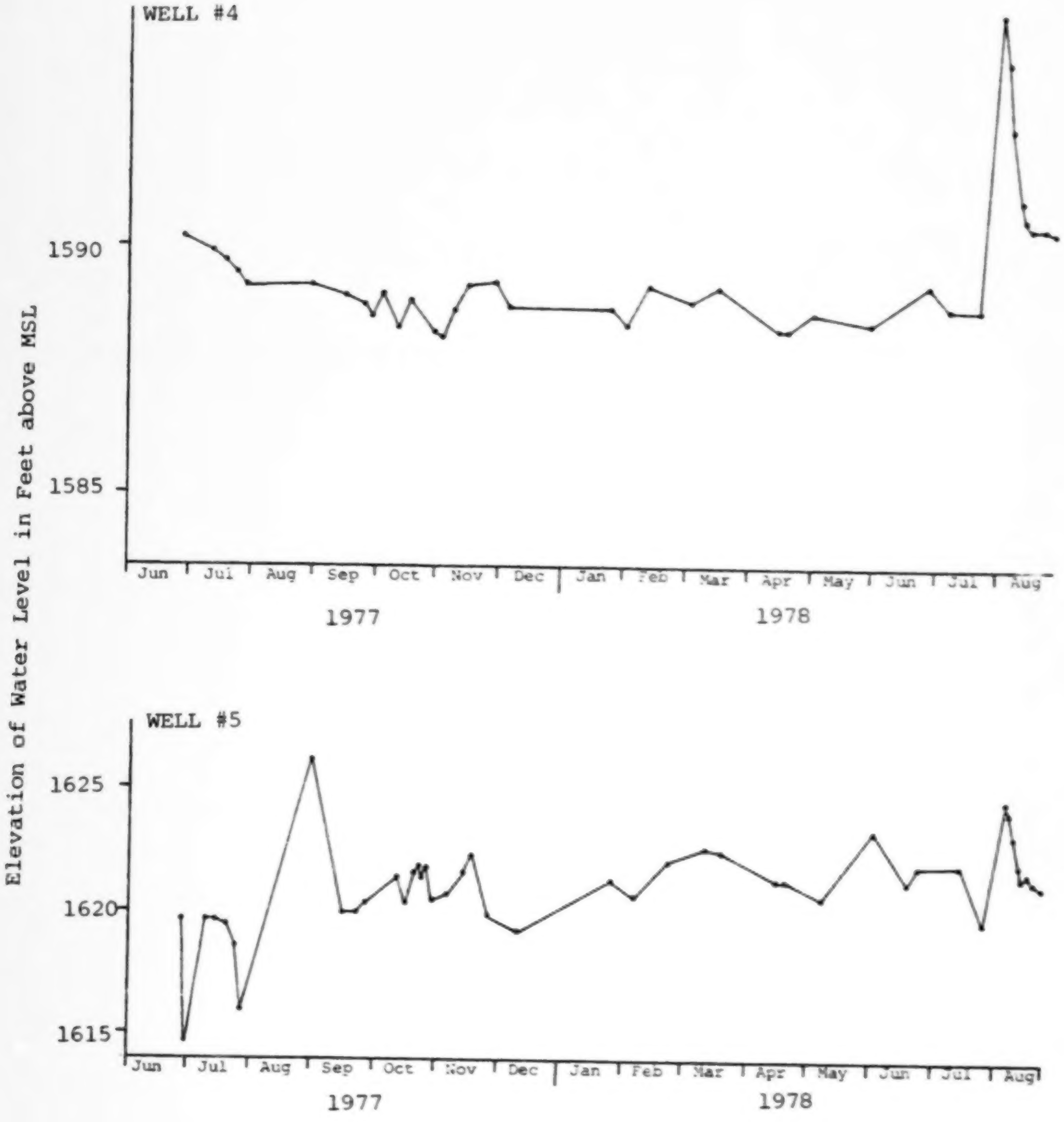

FIGURE 20. Hydrographs of Wells 4 and 5.

TABLE 49. Results of Chemical/Physical Analyses - Monitoring Wells

Parameter	Well #1		Well #2		Well #3		Well #4		Well #5		Irrigation Pond
	Sample	Mean/Range	Sample	Mean/Range	Sample	Mean/Range	Sample	Mean/Range	Sample	Mean/Range	Mean
Total Organic Carbon (mg/l)	13	11	18	8.6	24	<9.7 (<2 - 30)	25	12	9	6.9	21
Nitrate-Nitrogen (mg/l)	13	10.2	17	0.27	24	2.06	23	0.63	19	5.93	6.42
Nitrite-Nitrogen (mg/l as N)	13	1.50	18	<0.01-0.09	24	<0.01-0.06	23	<0.01-0.03	9	<0.01-1.90	1.95
Ammonia-Nitrogen (mg/l as N)	13	1.00	18	0.32	24	<0.01-0.08	25	<0.01-0.40	9	<0.01-0.39	2.91
Total Kjeldahl Nitrogen (mg/l)	13	2.50	18	0.57	24	0.38	25	<0.42 (<0.01-1.4)	9	0.68	6.20
Total Phosphate (mg/l as P)	13	<0.01-0.18	18	0.21	24	0.35	25	<0.13 (<0.01-0.37)	9	0.29	6.5
Ortho-phosphate (mg/l as P)	13	<0.01-0.09	18	<0.01-0.26	24	0.20	25	<0.01-0.64	9	0.18	5.4
Total Suspended Solids (mg/l)	5	17	11	73	12	24	13	21	7	36	39
Total Volatile Suspended Solids (mg/l)	5	5.6	11	26	12	<1.0-16	13	<1.0-11	7	6.0	30
Turbidity (NTU)	5	2.0	8	125	9	27	10	36	4	35	-
Conductivity (µmhos/cm)	5	1010	10	2600	11	1310	12	1340	6	1950	-
pH (units)	4	7.5	7	6.9	9	7.0	9	7.1	5	6.5	8.2

the material in which the wells are completed as well as the intermittent pumping for sampling. In fact, individual samples could be found to be as much as 100% in excess of the mean for both turbidity and total suspended solids. Under these cases (as with the mean values) only a small percentage of these solids were volatile.

Total organic carbon (TOC) levels were found to be highest in wells #1, 3 and 4. In the case of well #1, the influence of the landfill may be the cause of the elevated TOC (note this level was as high as that found in the lysimeters at 4.5 ft.). In the case of wells #3 and 4, the higher TOC levels can be attributed to the irrigation process and the direction of groundwater flow.

The influence of the landfill on well #1 may be evident in the metallic ion concentration data, also (see Table 50). Zinc and lead levels were found to be significantly higher in well #1 than in the other monitoring wells. The high levels of iron in well #3 were observed indirectly as an interference to other analyses. The fact that wells #2, 3 and 4 have higher iron concentrations is most likely reflective of the aquifer material in that part of alluvium. The calcium levels reflect the type material into which each of the wells was drilled.

Bacteriological Assay

Wells #1-4 at the Kerrville irrigation site were monitored routinely over a 13-month period beginning in July, 1977. Fewer samples were collected at well #5 due to a cement leak which produced high water pH (12-13). This problem was corrected in April, 1978, and regular sampling was initiated at that time.

During initial sampling at well #1 in July, 1977, false positive isolations for total coliform occurred on two occasions. Although green-pigmented colonies were observed, no positive fecal coliforms were detected in either sample. Subsequent biochemical characterizations resulted in the identification of these isolates as Pseudomonas species, possibly from the sanitary landfill located upslope of the well or from the groundwater itself.

Because of these early observations, limited laboratory testing was undertaken to verify the identity of organisms counted as fecal coliforms. Colonies were isolated from m-FC agar plates onto Heart Infusion Agar. Oxidase testing and a commercially-available identification system (Enterotube®, Roche Diagnostics) were employed to complete the speciation of the bacterial isolates. The results presented in Table 51 demonstrated the validity of the fecal coliform counts from well waters.

An overview of bacteriological results from all five monitoring wells is presented in Tables 52 and 53 as percentage of positive isolations and ranges of organism densities, respectively. As demonstrated by these data, water samples from all five wells showed evidence of fecal pollution. After its redevelopment (see above), well #5 consistently yielded water samples positive for all three bacterial indicators

TABLE 50. Groundwater Metallic Ion Concentration (mg/l)*

Well No.	Sample	Fe	Mg	Ca	K	Na
1	A	.43	10.20	124.0	12.51	50.0
	B	.31	9.69	118.0	12.21	48.0
2	A	4.17	57.0	97.50	14.49	132.0
	B	4.37	58.0	97.50	14.48	132.0
3	A	10.06	48.0	9[illegible].5	9.42	140.0
	B	11.96	47.25	95.0	9.15	145.0
4	A	8.34	39.25	97.50	8.17	156.25
	B	8.23	40.00	98.50	7.97	156.25
5	A	2.52	14.79	234.0	14.49	132.0
	B	1.69	15.81	216.0	14.48	132.0

Well No.	Sample	Cd	Zn	Mn	Pb	Cu	N	Cr
1	A	<0.027	0.39	0.02	1.73	0.03	<0.04	.01
	B	<0.027	0.12	<.02	1.12	<0.02	<0.04	.01
2	A	<0.027	0.021	0.267	<0.037	0.009	<0.26	<0.01
	B	<0.027	0.025	0.273				
3	A	<0.027	0.083	<0.037	<0.037	0.004	<0.26	<0.21
	B	<0.027	0.029	<0.037	<0.037	0.004	<0.26	<0.21
4	A	<0.027	0.020	0.277	<0.037	0.196	<0.26	<0.21
	B	<0.027	0.023	0.027	<0.037	0.007	<0.26	<0.21
5	A	<0.027	0.12	.06	<0.09	<0.02	<0.04	<0.01
	B	<0.027	0.12	.04	<0.09	<0.02	<0.04	<0.01

*Notes: Values are % recovered adjusted, except Cu and N; values reported as < are less than the detection limits based on three spiked distilled water samples.

TABLE 51. Identification of Fecal Coliform Isolates[†]

Sampling Date	Fecal Coliforms (cfu/100 ml)	No. of isolates	Identification
9/77	3.0×10^0	4	Klebsiella pneumoniae
		2	Enterobacter cloacae
1/78	8.7×10^0	10	Escherichia coli
		6	Klebsiella pneumoniae
		1	Citrobacter freundii
		1	Enterobacter cloacae

[†] Sample collected from Well #4, enumerated on M-FC agar as described previously.

TABLE 52. Percentage of Positive Isolations of Indicator Organisms from Kerrville Monitoring Wells

Well Number	Positive Samples (%) Total Coliform	Fecal Coliform	Fecal Streptococci
1	46	40	43
2	82	47	39
3	91	67	54
4	92	86	64
5	100	100	100

TABLE 53. Results of Bacterial Analyses, Kerrville Monitoring Wells[†]

Well Number	Total Coliform (cfu/100 ml)		Fecal Coliform (cfu/100 ml)		Fecal Streptococci (cfu/100 ml)	
	Median β	Range	Median β	Range	Median β	Range
1	$3.9x10^{1}$	$<.33 - 8.8x10^{1}$	$4.4x10^{0}$	$<.33 - 8.4x10^{1}$	$5.7x10^{1}$	$<.33 - 3.8x10^{2}$
2	$2.0x10^{1}$	$<.33 - 9.0x10^{1}$	$6.0x10^{0}$	$<.33 - 2.2x10^{1}$	$9.0x10^{0}$	$<.33 - 9.5x10^{2}$
3	$2.0x10^{0}$	$<.33 - 5.4x10^{2}$	$2.6x10^{0}$	$<.33 - 4.1x10^{2}$	$6.7x10^{-1}$	$<.33 - 1.5x10^{2}$
4	$2.6x10^{1}$	$<.33 - 1.1x10^{2}$	$2.4x10^{0}$	$<.33 - 5.3x10^{1}$	$2.8x10^{0}$	$<.33 - 3.6x10^{2}$
5[α]	$3.1x10^{3}$	$7.0x10^{1} - 1.0x10^{4}$	$3.7x10^{2}$	$3.0x10^{0} - 1.9x10^{3}$	$7.7x10^{1}$	$1.0x10^{0} - 3.9x10^{3}$

[†] sampling period 7/77 - 8/78

[α] sampling period 4/78 - 8/78

[β] median values derived from positive samples at densities exceeding those found in other wells. The upslope placement and depth of wells #1 and 5 coupled with the relatively lower organism densities in well #1 is consistent with a source of fecal pollution in well #5 from outside the irrigation site.

Of the remaining wells, #3 and 4 showed the greatest frequency of samples positive for fecal coliforms. Bacteriological data for total and fecal coliforms at well #2 were comparable to those observed at wells #3 and 4. Apparently, the effects of effluent irrigation on groundwater quality were at least as pronounced as any seepage from the irrigation pond (a result which would have been detected in well #2).

Results of well water analyses of samples taken with greater frequency over a two-week period are presented in Table 54. During this period, both wastewater irrigation and rainfall occurred. As noted earlier, wells #3 and 4 showed the greatest increase in both total and fecal coliforms with levels peaking on October 24 and 25, 1977. Within two days, the levels of fecal coliform had fallen to nondetectable levels in well #3 while densities in well #4 persisted at a low level of $2x10^0$ fecal coliform/100 ml six days later.

Viral Assays

During initial sampling conducted in July, 1977, well water samples were plated directly and after concentration (20-liter volumes) for detection of bacteriophage. Over a three-week period, wells #1, 3 and 4 were each sampled three times as described. A single coliphage plaque-forming unit was recovered from a concentrated sample collected at well #3 and another from a sample collected at well #4.

Because of the interest in detection of human enteric viruses in groundwater at an effluent irrigation site, the emphasis in field sampling was shifted to concentrating large volumes of well water for assay in primate cell cultures. Therefore, bacteriophage screening was discontinued except for occasional plating of extra concentrated volumes. No further coliphages were isolated. This was not unexpected as concentration procedures were optimized for enterovirus, not phage, recovery.

As enterovirus levels were extremely low in the irrigated wastewater, it was necessary to concentrate maximal volumes of well water. Wherever possible, the portable virus concentrator as described in Standard Methods, 14th edition, was utilized in monitoring well waters.

The greatest obstacle in routine viral testing was low water productivity in several wells. As indicated by sample sizes concentrated (Table 55), the yields from wells #1 and 2 were severely limited. Fortunately, both wells #3 and 4 which had shown the greatest influence of irrigation (a statement based on the bacteriological results cited earlier) were amenable to routine 100 gallon sampling.

Over a twelve-month study period (9/77-8/78), a total of nineteen separate large-volume samples (≥ 380 liters) were concentrated from both wells #3 and 4. Smaller volumes (≥ 20 liters) from wells #1 and 2 were

TABLE 54. Selected Bacteriological Results
Kerrville Monitoring Wells†

Well Number	Total Coliforms (cfu/100 ml)					
	10/13	10/20	10/24	10/25	10/27	10/31
1	$<3.3\text{x}10^{-1}$		$<3.3\text{x}10^{-1}$			$<3.3\text{x}10^{-1}$
2	$<3.3\text{x}10^{-1}$		$5.0\text{x}10^{0}$			$<3.3\text{x}10^{-1}$
3	$1.0\text{x}10^{0}$	$3.3\text{x}10^{-1}$	$2.6\text{x}10^{2}$	$4.3\text{x}10^{1}$	$1.0\text{x}10^{0}$	$1.0\text{x}10^{0}$
4	$2.2\text{x}10^{1}$	$8.3\text{x}10^{1}$	$6.0\text{x}10^{1}$	$5.5\text{x}10^{1}$	$3.0\text{x}10^{1}$	$1.5\text{x}10^{1}$

Well Number	Fecal Coliforms (cfu/100 ml)					
	10/13	10/20	10/24	10/25	10/27	10/31
1	$<3.3\text{x}10^{-1}$		$<3.3\text{x}10^{-1}$			$<3.3\text{x}10^{-1}$
2	$<3.3\text{x}10^{-1}$		$1.3\text{x}10^{0}$			$<3.3\text{x}10^{-1}$
3	$<3.3\text{x}10^{-1}$	$<3.3\text{x}10^{-1}$	$1.0\text{x}10^{0}$	$2.6\text{x}10^{1}$	$<3.3\text{x}10^{-1}$	$<3.3\text{x}10^{-1}$
4	$2.0\text{x}10^{0}$	$2.0\text{x}10^{1}$	$1.6\text{x}10^{1}$	$2.4\text{x}10^{1}$	$1.3\text{x}10^{1}$	$2.0\text{x}10^{0}$

Well Number	Fecal Streptococci (cfu/100 ml)					
	10/13	10/20	10/24	10/25	10/27	10/31
1	$<1.6\text{x}10^{-1}$		$<1.6\text{x}10^{-1}$			$<1.6\text{x}10^{-1}$
2	$<1.6\text{x}10^{-1}$		$<1.6\text{x}10^{-1}$			$<1.6\text{x}10^{-1}$
3	$<1.6\text{x}10^{-1}$	$1.6\text{x}10^{-1}$	$7.0\text{x}10^{-1}$	$2.1\text{x}10^{0}$	$1.6\text{x}10^{-1}$	$1.6\text{x}10^{-1}$
4	$<1.6\text{x}10^{-1}$	$2.6\text{x}10^{0}$	$6.0\text{x}10^{0}$	$3.8\text{x}10^{0}$	$3.0\text{x}10^{0}$	$3.3\text{x}10^{-1}$

† Week of 10/20/77; 3.2" rain, 1.9" wastewater irrigation;
Week of 10/27/77; 0.3" rain, 0.1" wastewater irrigation.

concentrated using diatomaceous earth, as described earlier, when water was available. Four samples ranging in size from 150 to 210 liters were concentrated from well #5.

TABLE 55. Volumes Routinely Concentrated for Enteroviruses from Wells

Well Number	Volumes Concentrated (liters)	
	Routinely	Maximally
1	20	40
2	20	50
3	380	630
4	380	760
5	150	210

A total of 21 potential viral isolates (as evidenced by any discernible areas of cpe) were picked from either HeLa or BGM cells during the course of this field monitoring program. Of this number, only two potential isolates from well #4 were passaged with production of cpe in homologous tube culture. However, the cpe attributable to both isolates could not be neutralized using the Lim-Benyesh-Melnick enterovirus pools (A-H).

An earlier passage of each isolate then was forwarded to a diagnostic virology laboratory (Texas Department of Health Resources) for isolation and identification. Although both *in vivo* and *in vitro* testing, specific for human enterovirus detection, were done, no viruses were isolated from either sample. Subsequently, several frozen (-76C) passages of both isolates were passaged again in the UTSA laboratory; however, no evidence of viral cpe could be detected.

As neither a positive identification nor a second recovery of viral infectivity could be achieved, no positive results for the well samples can be reported. This statement must be interpreted in light of the fact that positive viral isolations were made from water collected in soil at a depth of 4.5 feet (see lysimeters).

VIRAL RECOVERIES, IDENTIFICATION AND CHARACTERIZATION

Comparative Recoveries of Enteric Viruses on Primate Cell Lines

During the last year of field monitoring, assay procedures were modified in an attempt to increase viral detection sensitivity. Low passage Buffalo Green Monkey cells (BGM 110) were obtained from Dr. Gerald Berg, USEPA, Cincinnati, Ohio. Screening procedures for mycoplasma contamination were conducted routinely on this cell line with negative results. BGM cells along with HeLa cells were used in parallel plaque assay systems. In addition, BS-C-1 cells were used in a tube culture assay system for groundwater samples.

Data collected from these assay systems have been compiled to allow evaluation of relative viral detection. No positive viral isolations were made on the BS-C-1 cultures from either lysimeters or wells. After six weeks of intensive sampling at the Kerrville site, use of this system was discontinued. Representative comparative recoveries of indigenous viruses from different samples on the two cell lines used in the plaque assay system are shown in Table 56. HeLa monolayers yielded the highest number of isolates during this field study. On only two occasions were confirmed isolations made on BGM cells without parallel positive results on HeLa. On the other hand, it was not uncommon to observe positive viral isolations on HeLa cells in the absence of any recoveries in the BGM plaque assay system. Additionally, in the identification of isolates no viral types were unique to BGM cells, i.e., viruses isolated on BGM (polio 1) also were recovered on HeLa monolayers.

TABLE 56. Comparison of Indigenous Virus Recoveries (pfu) on HeLa and BGM Cells

Sample Source	Date of Sampling	Total pfu Observed*		Recovery Ratio
		HeLa	BGM	HeLa: BGM
Kerrville,				
Raw Wastewater	4/5/78	22	2	11
Raw Wastewater	8/14/78	6	8	0.8
Irrigation Pond	5/1/78	21	11	1.9
Lysimeter	5/1/78	7	4	1.8
Uvalde STP	6/14/78			
Raw Wastewater		484	30	16
Trickling Filter Effluent		485	24	20
Pond 1 Effluent		850	16	53
Pond 2 Effluent		588	14	42

* Equal volumes plated.

Viral Identification and Characterization

After the first year of plant monitoring, it became apparent that due to treatment effectiveness, only low levels of enteroviruses were being discharged into the irrigation system. Therefore, rigorous procedures were instituted for reporting the presence of viruses in pondwaters, surface water, groundwater, and soils. Isolates which could not be passaged in a homologous tube culture system were not reported. Further, every attempt was made to identify confirmed viruses by conventional serum neutralization procedures. In most cases, viruses were identified successfully, although mixed samples containing more than one virus undoubtedly contributed to the nonidentification of some isolates.

Results shown in Table 57 summarize the identified viral types recovered on both HeLa and BGM cell lines from the Kerrville irrigation site during the last two years. The predominant isolates were categorized as type 1 poliovirus. Of the total number of confirmed viruses from the irrigation pond and lysimeter waters, poliovirus 1 accounted for 87% and 67% of the isolates respectively. Coxsackievirus B and ECHO viruses also were recovered from these sources.

Because of the large number of viral isolates identified as poliovirus 1 using the Lim-Benyesh-Melnick enterovirus typing pools, further characterization of these isolates was undertaken. Representative results from thermal exposure testing are presented in Table 58. Visual inspection of calculated r values indicate that of the field isolates tested, 75% showed no reduction in titer as a result of incubation at 40C. Laboratory seed virus, poliovirus 1 (Chat), responded as expected showing a 1 $\log_{10}$ reduction while a reference strain of wild type poliovirus 1 (Mahoney) showed no plaque reduction at elevated incubation temperature.

Obviously, these results must be interpreted with care. No single marker can be used to define a strain as the attenuated vaccine or wild-type virus. In addition, reversion of the poliovirus temperature sensitivity marker has been noted. Nevertheless, these results from rct testing strongly suggest that the isolates recovered from field samples were not identical with the laboratory seed virus, poliovirus 1 (Chat).

The implications of the isolation from any single water sample of virions whose rct behavior parallels a reference wild-type should not be exaggerated. In no way does it constitute evidence of either a reservoir of wild-type poliovirus in the general population or selective survival of wild-type over attenuated virions. The relative prevalence of wild-type and attenuated poliovirus in the raw wastewater at Kerrville was not determined. In the absence of this information and of more definitive markers, these data can simply be construed as suggestive.

TABLE 57. Identification of Viral Isolates from the Kerrville Irrigation Site

Sample Source	Date of Isolation	Viral Identification	Number of Isolates
Lysimeters			
1.5 feet	2/23/77	polio 1	1
	8/2/78	Coxsackie B4	1
		Coxsackie B5	2
3.0 feet	10/26/77	Coxsackie B5	1
	1/3/78	ECHO 21	1
		ECHO 11	1
	5/1/78	polio 1	1
	8/2/78	Coxsackie B4	1
4.5 feet	2/18/77	polio 1	5
	5/1/78	polio 1	11
	8/2/78	Coxsackie B4	1
		Coxsackie B3	1
Irrigation Pond	5/9/77	polio 1	4
	11/3/77	polio 1	1
		Coxsackie B5	4
	5/1/78	polio 1	21
Soils	2/28/77	polio 1	1
	5/2/78	polio 1	1
Third Creek, Upstream	5/9/77	polio 1	3

TABLE 58. Temperature Characterization of Poliovirus 1 Isolates, Kerrville Irrigation Site

Sample Source	titer (pfu/ml) 37C	titer (pfu/ml) 40C†	r Value*
Soil, 5/2/78	$5.5\text{x}10^7$	$6.0\text{x}10^7$	1.09
Lysimeter, 5/1/78			
K-314	$5.3\text{x}10^7$	$5.5\text{x}10^7$	1.04
K-315	$6.6\text{x}10^7$	$9.3\text{x}10^7$	1.40
K-316	$5.2\text{x}10^7$	$6.0\text{x}10^7$	1.15
K-317	$4.5\text{x}10^7$	$4.7\text{x}10^6$	0.10
K-318	$5.3\text{x}10^7$	$7.2\text{x}10^6$	0.14
K-319	$8.8\text{x}10^7$	$9.8\text{x}10^7$	1.11
K-320	$9.1\text{x}10^7$	$9.0\text{x}10^7$	0.99
Poliovirus 1 (Chat)	$6.2\text{x}10^8$	$6.9\text{x}10^7$	0.11
Poliovirus 1 (Mahoney)	$2.7\text{x}10^8$	$3.3\text{x}10^8$	1.22

† 40.1 ± 0.2C

* $r = \frac{\text{Total number of plaques observed at 40C}}{\text{Total number of plaques observed at 37C}}$

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APPENDIX A

Viral Identification and Characterization

Isolate No.	Sample Source	Date of Isolation	Cell Line	Viral Identification	Poliovirus 1 Characterization r#
S-8	Lysimeter 2-B	2/18/77	HeLa	Polio 1	
S-9	Lysimeter 2-B	2/18/77	HeLa	Polio 1	
S-10	Lysimeter 2-B	2/18/77	HeLa	Polio 1	
S-11	Lysimeter 2-B	2/18/77	HeLa	Polio 1	
S-12	Lysimeter 2-B	2/18/77	HeLa	Polio 1	
S-13	Lysimeter 2-B	2/18/77	Mixed Culture	Possibly Polio 1 + ECHO 21	
S-14	Lysimeter 3-T	2/23/77	HeLa	Polio 1	
S-18	Soil #1	2/28/77	HeLa	Polio 1	
K-9	Lysimeter 1-M	10/26/77	HeLa	Coxsackie B5	
K-43	Third Creek Up	5/9/77	HeLa	Polio 1	
K-45	Third Creek Up	5/9/77	HeLa	Polio 1	
K-46	Third Creek Up	5/9/77	HeLa	Polio 1	
K-306	Irrigation Pond	5/9/77	HeLa	Polio 1	
K-307	Irrigation Pond	5/9/77	HeLa	Polio 1	
K-308	Irrigation Pond	5/9/77	HeLa	Polio 1	
K-309	Irrigation Pond	5/9/77	HeLa	Polio 1	
K-50	Irrigation Pond	11/3/77	HeLa	Polio 1	
K-52	Irrigation Pond	11/3/77	HeLa	Coxsackie B5	
K-54	Irrigation Pond	11/3/77	HeLa	Coxsackie B5	
K-55	Irrigation Pond	11/3/77	HeLa	Coxsackie B5	
K-56	Irrigation Pond	11/3/77	HeLa	Coxsackie B5	
K-67	Lysimeter 1-M	1/31/78	HeLa	ECHO 21	
K-68	Lysimeter 1-M	1/31/78	HeLa	ECHO 11	
K-310	Soil @ Lys. 2	5/2/78	HeLa	Polio 1	1.09
K-314	Lysimeter 2-B	5/1/78	HeLa	Polio 1	1.04
K-315	Lysimeter 2-B	5/1/78	HeLa	Polio 1	1.4
K-316	Lysimeter 2-B	5/1/78	HeLa	Polio 1	1.15
K-317	Lysimeter 2-B	5/1/78	HeLa	Polio 1	0.104
K-318	Lysimeter 2-B	5/1/78	HeLa	Polio 1	0.14
K-319	Lysimeter 2-B	5/1/78	HeLa	Polio 1	1.1
K-320	Lysimeter 2-B	5/1/78	HeLa	Polio 1	0.99
K-347	Lysimeter 2-M	5/1/78	BGM	Polio 1	
K-349	Lysimeter 2-B	5/1/78	BGM	Polio 1	
K-350	Lysimeter 2-B	5/1/78	BGM	Polio 1	
K-351	Lysimeter 2-B	5/1/78	BGM	Polio 1	
K-352	Lysimeter 2-B	5/1/78	BGM	Polio 1	
K-321	Irrigation Pond	5/1/78	HeLa	Polio 1	
K-322	Irrigation Pond	5/1/78	HeLa	Polio 1	
K-323	Irrigation Pond	5/1/78	HeLa	Polio 1	0.7
K-324	Irrigation Pond	5/1/78	HeLa	Polio 1	0.78
K-325	Irrigation Pond	5/1/78	HeLa	Polio 1	
K-326	Irrigation Pond	5/1/78	HeLa	Polio 1	
K-327	Irrigation Pond	5/1/78	HeLa	Polio 1	
K-328	Irrigation Pond	5/1/78	HeLa	Polio 1	
K-329	Irrigation Pond	5/1/78	HeLa	Polio 1	1.26

# r = total number of pfu observed at 40C: total number observed at 37C.

APPENDIX A (continued)

Viral Identification and Characterization

Isolate No.	Sample Source	Date of Isolation	Cell Line	Viral Identification	Poliovirus 1 Characterization r#
K-330	Irrigation Pond	5/1/78	HeLa	Polio 1	0.9
K-331	Irrigation Pond	5/1/78	HeLa	Polio 1	
K-332	Irrigation Pond	5/1/78	HeLa	Polio 1	0.84
K-333	Irrigation Pond	5/1/78	HeLa	Polio 1	1.0
K-334	Irrigation Pond	5/1/78	HeLa	Polio 1	1.02
K-335	Irrigation Pond	5/1/78	HeLa	Polio 1	
K-336	Irrigation Pond	5/1/78	HeLa	NI*	
K-337	Irrigation Pond	5/1/78	HeLa	NI	
K-338	Irrigation Pond	5/1/78	HeLa	NI	
K-339	Irrigation Pond	5/1/78	HeLa	NI	
K-340	Irrigation Pond	5/1/78	HeLa	NI	
K-341	Irrigation Pond	5/1/78	HeLa	NI	
K-353	Irrigation Pond	5/1/78	BGM	Polio 1	
K-354	Irrigation Pond	5/1/78	BGM	NI	
K-355	Irrigation Pond	5/1/78	BGM	Polio 1	
K-356	Irrigation Pond	5/1/78	BGM	Polio 1	
K-357	Irrigation Pond	5/1/78	BGM	NI	
K-358	Irrigation Pond	5/1/78	BGM	Polio 1	
K-359	Irrigation Pond	5/1/78	BGM	NI	
K-360	Irrigation Pond	5/1/78	BGM	NI	
K-362	Irrigation Pond	5/1/78	BGM	Polio 1	
K-363	Irrigation Pond	5/1/78	BGM	Polio 1	
K-364	Irrigation Pond	5/1/78	BGM	NI	
K-371	Lysimeter 1-T	8/2/78	BGM	NI	
K-373	Lysimeter 2-B	8/2/78	HeLa	Coxsackie B4	
K-374	Lysimeter 2-M	8/2/78	HeLa	Coxsackie B4	
K-375	Lysimeter 2-T	8/2/78	HeLa	Coxsackie B4	
K-377	Lysimeter 2-T	8/2/78	HeLa	Coxsackie B5	
K-378	Lysimeter 2-B	8/2/78	HeLa	Coxsackie B3	
K-379	Lysimeter 2-T	8/2/78	HeLa	Coxsackie B5	
K-385	Irrigation Pond Sediment	9/13/78	HeLa	Coxsackie B5	
K-47	Third Creek Down	11/25/77	HeLa	NI	

#r = total number of pfu observed at 40C: total number observed at 37C.

*NI = virus confirmed by tube culture passage but not identified.

APPENDIX B

Growth of Bacterial Indicators in Lysimeter Waters

Lysimeter, 1.5 feet (pH 7.4)

Sampling Time (days)	Temperature					
	4C		20C		35C	
	cfu/100ml	N/No	cfu/100ml	N/No	cfu/100ml	N/No
Total Coliforms						
1	$6.1x10^{4}$	$1.0x10^{0}$	$6.1x10^{4}$	$1.0x10^{0}$	$6.1x10^{4}$	$1.0x10^{0}$
2	$1.8x10^{4}$	$3.0x10^{-1}$	$1.9x10^{4}$	$3.1x10^{-1}$	$8.2x10^{4}$	$1.3x10^{0}$
3	$6.7x10^{4}$	$1.1x10^{0}$	$3.2x10^{4}$	$5.2x10^{-1}$	$3.3x10^{4}$	$5.4x10^{-1}$
4	$9.7x10^{4}$	$1.6x10^{0}$	$5.7x10^{4}$	$9.3x10^{-1}$	$6.0x10^{3}$	$9.8x10^{-2}$
5	$8.1x10^{4}$	$1.3x10^{0}$	$3.2x10^{4}$	$5.2x10^{-1}$	$1.4x10^{3}$	$2.3x10^{-2}$
6	$7.2x10^{4}$	$1.2x10^{0}$	$1.3x10^{4}$	$2.1x10^{-1}$	$6.1x10^{2}$	$1.0x10^{-2}$
7	$8.7x10^{4}$	$1.4x10^{0}$	$8.8x10^{3}$	$1.4x10^{-1}$	$4.1x10^{2}$	$6.7x10^{-3}$
10	$8.2x10^{4}$	$1.3x10^{0}$	$4.1x10^{3}$	$6.7x10^{-2}$	$1.2x10^{2}$	$2.0x10^{-3}$
Fecal Streptococci						
1	$4.5x10^{3}$	$1.0x10^{0}$	$4.5x10^{3}$	$1.0x10^{0}$	$4.5x10^{3}$	$1.0x10^{0}$
2	$3.0x10^{3}$	$6.7x10^{-1}$	$4.2x10^{3}$	$9.3x10^{-1}$	$6.7x10^{3}$	$1.5x10^{0}$
3	$3.5x10^{3}$	$7.8x10^{-1}$	$1.2x10^{3}$	$2.7x10^{-1}$	$7.8x10^{2}$	$1.7x10^{-1}$
4	$3.3x10^{3}$	$7.3x10^{-1}$	$9.1x10^{2}$	$2.0x10^{-1}$	$9.0x10^{1}$	$2.0x10^{-2}$
5	$2.6x10^{3}$	$5.8x10^{-1}$	$3.5x10^{2}$	$7.8x10^{-2}$	$3.3x10^{1}$	$7.3x10^{-3}$
6	$1.7x10^{3}$	$3.8x10^{-1}$	$1.4x10^{2}$	$3.1x10^{-2}$	1.3x10	$2.9x10^{-3}$
7	$1.3x10^{3}$	$2.9x10^{-1}$	$6.7x10^{1}$	$1.5x10^{-3}$	$6.7x10^{0}$	$1.5x10^{-3}$
10	$1.3x10^{3}$	$2.9x10^{-1}$	$1.7x10^{1}$	$3.8x10^{-3}$	$6.7x10^{0}$	$1.5x10^{-3}$
Total Coliforms						
1	$3.2x10^{4}$	$1.0x10^{0}$	$3.2x10^{4}$	$1.0x10^{0}$	$3.2x10^{4}$	$1.0x10^{0}$
2	$1.6x10^{4}$	5.0x10-1	$1.5x10^{4}$	4.7x10-1	$3.7x10^{4}$	$1.2x10^{0}$
3	$1.5x10^{4}$	$4.7x10^{-1}$	$1.0x10^{4}$	$3.1x10^{-1}$	$1.2x10^{4}$	$3.8x10^{-1}$
4	$3.8x10^{4}$	$1.2x10^{0}$	$2.3x10^{4}$	$7.2x10^{-1}$	$7.2x10^{3}$	$2.3x10^{-1}$
5	$1.5x10^{4}$	$4.7x10^{-1}$	$8.4x10^{3}$	$2.6x10^{-1}$	$2.7x10^{3}$	$8.4x10^{-2}$
6	$1.3x10^{4}$	$4.1x10^{-1}$	$6.3x10^{3}$	$2.0x10^{-1}$	$7.3x10^{2}$	$2.3x10^{-2}$
7	$1.1x10^{4}$	$3.4x10^{-1}$	$7.6x10^{3}$	$2.4x10^{-1}$	$4.1x10^{2}$	$1.3x10^{-2}$
10	$9.9x10^{3}$	$3.1x10^{-1}$	$4.3x10^{3}$	$1.3x10^{-1}$	$7.7x10^{1}$	$2.4x10^{-3}$
Fecal Streptococci						
1	$1.5x10^{3}$	$1.0x10^{0}$	$1.5x10^{3}$	$1.0x10^{0}$	$1.5x10^{3}$	$1.0x10^{0}$
2	$1.1x10^{3}$	$7.3x10^{-1}$	$3.0x10^{2}$	$2.0x10^{-1}$	$9.8x10^{2}$	$6.5x10^{-1}$
3	$6.3x10^{2}$	$4.2x10^{-1}$	$1.8x10^{2}$	$1.2x10^{-1}$	$2.4x10^{2}$	$1.6x10^{-1}$
4	$3.1x10^{2}$	$2.1x10^{-1}$	$1.0x10^{2}$	$6.7x10^{-2}$	$1.7x10^{1}$	$1.1x10^{-2}$
5	$8.3x10^{1}$	$5.5x10^{-2}$	$8.7x10^{1}$	$5.8x10^{-2}$	$3.3x10^{0}$	$2.2x10^{-3}$
6	$6.0x10^{1}$	$4.0x10^{-2}$	$8.7x10^{1}$	$5.8x10^{-2}$	$6.7x10^{0}$	$4.5x10^{-3}$
7	$6.0x10^{1}$	$4.0x10^{-2}$	$6.0x10^{1}$	$4.0x10^{-2}$	$1.3x10^{1}$	$8.6x10^{-3}$
10	$3.7x10^{1}$	$2.5x10^{-2}$	$1.0x10^{1}$	$6.7x10^{-3}$	$6.7x10^{0}$	$4.5x10^{-3}$